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ARE THE FACTORS OF HEREDITY ARRANGED IN A LINE?

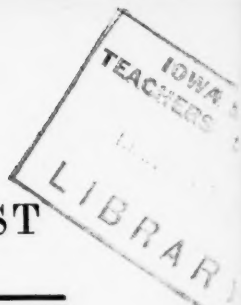
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IN the February (1919) number of the *Proceedings of the National Academy of Sciences*, Professor Castle states that he has "shown that the arrangement of the genes in the sex-chromosome of *Drosophila ampelophila* is probably not linear, and a method has been developed for constructing a model of the experimentally determined linkage relationships."¹ This declaration is so widely at variance with the conclusions jointly agreed upon by all *Drosophila* workers, that the arguments or assumptions which it involves would seem to call for careful examination. It may be stated at the outset that the principle upon which Professor Castle constructs his models appears exceedingly direct and simple—it is merely to make a figure such that the distances between all the points represented on it are exactly proportional to the frequencies of separation actually found between the respective factors in the most reliable experiments. If this is done, Castle contends, the models will be three-dimensional instead of linear in shape.

1. The first argument which Castle gives against the view that the groups of genes (which he admits, at least

¹ Sturtevant, Bridges and Morgan also have published a defense of the view of linear linkage, in the *Proceedings of the National Academy of Sciences* (5, 1919, pp. 168-173) and Professor Castle has just replied to them in the same journal (5, 1919, pp. 501-506). It is believed that the present paper, although written and accepted for publication in the *NATURALIST*, previously to this article, meets all the points therein brought forward.



for purposes of argument, to be in the chromosomes) are linear, is that "it is doubtful . . . whether an elaborate organic molecule ever has a simple string-like form." This argument is therefore based upon the unique assumption that the whole chromosome (or that part of it containing the genes) consists of one huge molecule. Later, he speaks still more explicitly of this "chromosome molecule" and says, "the duplex linkage systems of a germ cell at the reduction division must be . . . twin organic molecules," so that "a purely mechanical theory [of crossing over] seems inadequate to account for interchange of equivalent parts between them." The argument may therefore be paraphrased as follows: since (1) the whole group of genes is but a single organic molecule, and since (2) an organic molecule can not be linear, then it must follow that (3) the group of genes is not linear, and that the theory of crossing over is therefore erroneous. Although the premises of this argument are both entirely gratuitous, it must be admitted that there is no flaw in the reasoning, once the premises are admitted.

2. The second argument brought forward against the linear arrangement of genes is that, in the linear maps, the distances between widely separated loci are not strictly proportional to the per cents of crossing over actually found, being relatively too large, in comparison with the per cents of crossing over. This he terms a "discrepancy" in the map, which has required the "subsidiary hypothesis" of double crossing over, in order to harmonize it with the theory of linear linkage. The answer to this is that it has never been claimed, in the theory of linear linkage, that the per cents of crossing over are actually proportional to the map distances: what has been stated is that the per cents of crossing over are *calculable* from the map distances—or, to put the matter in more mathematical terms, that the per cents of crossing over are a *function* of the distances of points from each other along a straight line. As will be shown presently, this circumstance alone is sufficient to

show that the factors must be bound together in a linear series; the precise nature of the function (involving coincidence, etc.) will then determine for us precisely the mode of incidence of the crossing over—i. e., granted the linear series, it is then possible to calculate from the data the exact frequency of single crossing over, double crossing over of the various possible types, and multiple crossing over. Double crossing over thus becomes, not a “subsidiary hypothesis,” but a phenomenon directly demonstrated.

It may, however, be noted in passing that, even if there had been no experimental evidence at all in regard to the nature of the linkage it could not have been conceded that Castle's alternative postulate—that no double crossing over can ever occur at all—would have been any more plausible *a priori* than that of the *Drosophila* workers which admits the existence of double crossing over. For, once the occurrence of single breaks in a chromosome is admitted—a point agreed upon by both sides—it is just as arbitrary to deny the possibility of double breaks as to assert their existence. Although Castle nowhere does explicitly admit that he has adopted this alternative “subsidiary hypothesis”—the denial of the possibility of double crossing over—yet an inspection of the theory of linkage which he himself has proposed shows that in this it has been tacitly assumed throughout, being necessary for the purposes of the solid models. Were double crossing over once admitted to occur, it could no longer be claimed that the distances between the factors in the three-dimensional models are exactly proportional to their per cents of separation—a condition which it is the sole aim of the existence of the models to fulfill.

We may now return to examine more carefully the main argument upon which linear arrangement and its corollaries (double crossing over, coincidence, etc.) is based. The fact previously stated that the linkage relations between the genes are such that they are all calculable from the positions of points in a linear series

may also be expressed as follows: given any three linked factors, A, B and C, if any two of the linkages between them are known—say, the linkage AB and BC—then the third linkage—AC—is determined (the most convenient practical method for calculating it is to make use of the “curve of coincidence” of the particular chromosome). This is, two of the linkage values may be taken as “independent variables” and the third is then “dependent” on them—in this sense we may say that B is linked directly to A and to C, but that A is only linked to C through the linkage of each of these factors with B. Since this is true of any combination of three-linked factors (ABC, BCD, CDE, ACD, etc.) it can be shown that the factors are all linked together in chain arrangement, any one factor being linked directly to only two others (those which we may regard as being on either side of it), its linkage with the rest being entirely dependent on these intermediary linkages.¹ This remains true as a discovered mathematical fact of the linkage relationships, shown first in experiments of Sturtevant’s designed to investigate the problem, and this is what the writer has designated as “the law of linear linkage.” Whether or not we regard the factors as lying in an actual material thread, it must on the basis of these findings be admitted that the forces holding them linked together—be they physical, “dynamic” or transcendental—are of such a nature that each factor is directly

¹I. e., all the linkages (factorial $(n-1)$ in number) between the n factors in a group, can be shown to be dependent on (functions of) only $n-1$ “primary” or “independent” linkages. To obtain the most perfect expression of this dependency it is necessary to choose as the $n-1$ independent values the two strongest linkages involving each of the n factors (what we should call linkages AB, BC, CD, DE, etc., as contrasted with AC, AD, AE, BD, etc.). On this system, the other linkages all become definitely determined, the secondary linkages being in each case a function involving the sum of certain of the primary linkages. If, however, the primary linkages are not chosen according to the above rule, so as to constitute a “chain formation,” no formula can definitely express the relationships of the linkages, for the secondary linkages will then in some cases depend upon the sum, in other cases upon the difference between the linkages taken as primary.

bound, in segregation, with only two others—in bipolar fashion—so that the whole group, dynamically considered, is a chain. This does not necessarily mean that the spatial relations of the factors accord with these dynamic relations, for it is conceivable *a priori* that factor A might be far off from B, in another part of the cell, or that both might be diffused throughout the cell, and that they might nevertheless attract each other, during the segregation division, by some sort of chemical or physical influence. In the discussion that follows, no implication as to the actual physical arrangement of the genes is intended when the terms “linear series,” “distance,” etc., are used; these will refer only to the relations existing between the points in the linear map, which may be regarded merely as a mathematical mode of representation of the data themselves. It will be shown, however, at the conclusion of this article, that when the various conditions which have to be fulfilled at segregation are taken into consideration, any other explanation for these peculiarly linear linkage findings than an arrangement of the genes in a spatial, physical line proves to be haz- ardously fanciful.

In the case of the larger distances, in order to discover what function of the distance the per cent. of separation represents, it would be necessary to conduct extremely delicate determinations, involving very extensive data in experiments dealing with many points simultaneously. Nevertheless enough has been done to show that even for the larger distances the per cents of separation do depend on the distance in the linear map—being less than the distance by an amount which varies in a fairly regular manner according to the distance itself; hence it is known that the higher per cents of separation certainly involve some function of the linear distance.

In the case of the smaller distances, on the other hand, the function has been rather accurately ascertained; it is very close to the simplest one possible, that is, there is an almost exact proportionality here between the map

distances and the per cents of separations. Just as distance AB plus distance BC on a line are equal to distance AC, so the corresponding small frequency of separation between A and B, plus the small frequency between B and C, are found to be almost exactly equal to the frequency of separation between A and C; for this reason if the factors A, B and C are represented as points in a straight linear map, the distances between any two of them will represent the corresponding separation frequencies in an almost proportionate manner. A few examples of this principle are shown in Table I; it has been confirmed in innumerable other crosses, with many different factors. Moreover, it is found that the smaller the distance involved, the more exact is the proportionality that obtains, the less being the relative discrepancy between the frequency AC as found by experiment, and the value AC obtained, as on the map, by the summation of values AB and BC. The relationship which exists between the small separation values is hence just the sort which Castle himself would demand, for a proof of linear linkage. But whereas Castle would require this relationship to hold for all values, small or large, it may be shown that its existence in the case of the small alone is all that would be necessary for a complete proof of the doctrine of linear linkage, even if the large values were no sort of function of the linear series. For, if we proceed according to Castle's own method, and construct a map to represent the relations of the small values just described, showing each of the frequencies by a proportionate distance on the map, we necessarily obtain a map each section of which is practically a straight line. In the case, for example, of the data for *v*, *g*, and *f*, shown in Table I, if we represent the separation frequencies by proportionate distances in space, we must place point *v* at 10.7 units from *g*, and *g* at 11.3 units from *f*; if these two conditions are both to hold, then the only possible way of bringing *f* to its distance of 21.8 units from *v* is to put the three points in a nearly straight line, as shown

TABLE I

BACK CROSS OF FEMALES HETEROZYGOUS FOR *v*, *g* AND *f*. (PERFORMED BY BRIDGES; REPORTED BY WEINSTEIN)

I. Non-separations	II. Separations of <i>v</i> from <i>g</i> and <i>f</i>	III. Separations of <i>f</i> from <i>v</i> and <i>g</i>	IV. Separations of <i>g</i> from <i>v</i> and <i>f</i>	V. Total Files
2651	360	380	3	3394
Resultant Per Cent. of Separations Between <i>v</i> and <i>g</i>	Resultant Per Cent. of Separations Between <i>g</i> and <i>f</i>	Resultant Per Cent. of Separations Between <i>v</i> and <i>f</i>		
$\frac{(II+IV)}{V}$	$\frac{(III+IV)}{V}$	$\frac{(II+III)}{V}$		
10.7	11.3	21.8		

in Fig. 1. Other results indicate that the line would be exactly straight if still smaller distances were studied. Enough data have been obtained in the case of chromosome I of *Drosophila* to determine in this way the "shape" of each part of the linkage group, and each part, by itself, is thus found to follow the rules for linear

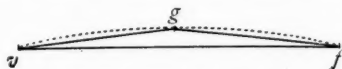


FIG. 1. Direct representation of the linkages in Table I. (*vg*, *gf*, and *vf* are each represented by a line of length proportionate to the respective frequency of separation.) The dotted curve shows the "average angular deviation" of the factors from a straight line.

distances in an extraordinarily rigorous manner. That is, given the factors ABCDE, etc.,—or to take an actual case, *y*, *w*, *A*, *bi*, *cl*,—it is found that the linkages of *y*, *w*, and *A* are proportional to their distances in a straight line, so are the linkages of *w*, *A*, and *bi*, for *A*, *bi*, and *cl*, etc. But, since every part of the group is thus linear, it must then be true that the entire group is linear. A line all of the parts of which are straight is a straight line. Any differences then observed between the size of the larger distances and the per cents of crossing over, even if they were so irregular that they could not be thought of as a function of the linear system itself, would then have to be regarded as due to peculiarities in the incidence of the crossing over, superimposed upon a system of genes which was really linear in formation,—

modifications due to specific correlations between crossings over in different regions. But since, as has been stated, the differences between the larger per cents of crossing over and the linear distances are not unregulated, but do give clear evidence of being themselves a function of the map distances, these larger per cents of separation as well as the smaller ones can be used in proof of the linear system of linkage. The systematic differences between the frequencies and the map are hence due to double and other multiple cross overs, which vary in frequency in accordance with the distance involved.

It is true that a certain amount of the differences actually found between the larger frequencies and their corre-

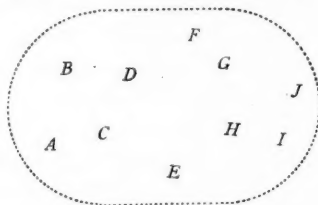


FIG. 2. Example of the sort of relations which would hold between factors arranged non-linearly (although in a plane figure). It is evident from this figure that more remote factors, such as *AE* and *J*, are likely to be arranged more nearly in a straight line than factors nearer together, such as *AB* and *C*.

sponding distances on a straight linear map might be thought of as due to the cumulation of minor discrepancies which existed between the small frequencies and distances but each one of which was by itself too small to be detected in the data for the smaller distances, being within the limits of experimental error. As the small discrepancies in such a case would, to be always cumulative, all have to have a bias in the same direction, this would amount to saying that the line along which the points were really disposed had a slow, even curve, too slight to be detected except when large distances were considered. The straight line would then be sufficiently accurate as a proportionate representation of all the

small values but not of the large ones. If the validity of the evenly curved figure were accepted, it would in no way disagree with the finding of a linear arrangement of the genes, but would merely substitute a curved line for a straight one. In a really non-linear figure, such as shown in Fig. 2, the relations between the smaller distances would be (if anything) less of a linear type than the relations between the larger distances,—factors further apart in a thick rod, for example, would have to be more in line than those near together. The fact that the opposite relation holds in the actual data shows conclusively that the factors are in some sort of a line. There is an *a priori* objection, however, to accepting a curved line as an explanation of the linkage relations, in that it is very difficult to imagine a plausible set of conditions in the chromosome which would hold the factors rigidly in this curved line but which would at the same time determine the number of separations between the factors according to their direct (straight) distances from each other, instead of according to their distances along this line. But, quite aside from *a priori* reasons, there is an experimental result absolutely fatal to the curved line "explanation"; this consists in the finding of those classes which are termed by the *Drosophila* workers "triple crossovers." In the case of these classes the separations are of such a type as to require the assumption of a break in the curved line at three points simultaneously. As it is obvious that a break in only one plane could not cut the curve at more than two points, the triple crossovers therefore would have to be due to a break in more than one plane. The occurrence of breaks in more than one plane, however, disturbs the assumed relation of simple proportionality between separation frequencies and map distances, which was the basic postulate upon which the curved line was constructed. If the distances are after all once admitted to be not exactly proportional to the separation frequencies, then there remains no reason to assume, just because the larger separation fre-

quencies are out of exact proportion to the distances on a straight linear map, that this is because the line is curved, and the factors thus nearer together. If still more evidence against the curved line idea be desired, it may be added that when the curve is constructed so as to be in good agreement (statistically) with the relations found between the smaller frequencies, it is then not sufficiently arched to permit the representation of the larger frequencies by proportionate distances (see section 4). A detailed compilation which I have made of all the data has shown that experimental error will not well account for the differences thus obtained between the two sets of results. The curved line being abandoned, it becomes, therefore, necessary to revert to "double crossing over," in explanation of the deviation of the large frequencies from the straight map values.²

3. If we examine further into Castle's argument, however, we find that he objects, not only because the larger separation frequencies are not proportional to the distances in linear maps, but also because he believes that the smaller frequencies are not proportional; in fact, according to his solid models, none of the kinds of frequencies, small or large, could even be a function of the distances in a linear map. In his solid, or rather, three-dimensional, models, which purport to have the factors so spaced that all distances between them are exactly proportional to the corresponding linkages, the factors are scattered about at all angles to each other, in such a

² The fact that the *geometrical* line which represents the linkages of the factors should be taken as straight does not imply that the supposed *physical* line in which the factors lie is straight. So long as the factors lie in any kind of physical line at all, then, if their linkages are determined, in some way, by their distances as measured *along* this line, these linkages should be representable on the basis of a straight geometrical map, inasmuch as all distances taken *along* a curved line must have the same interrelationships as distances in a straight line. Hence the curving of the chromosome filament is a matter entirely aside from the issue here involved, since the separation frequencies of the factors in the supposed filament are not conceived of as dependent upon their direct distances from each other but rather upon their distances along this filament. Thus the filament may, for these purposes, be treated as if it were straight.

way that their distances could never be represented as a function of distances in a single line. The cause of this discrepancy between Castle's figures and the relationships observed by the *Drosophila* workers lies in the nature of the data which Castle uses, or rather, in his manner of using the data. For Castle constructs his maps, or models, on all the data obtainable, indiscriminately, and regardless of the fact that most of the data for the linkage values involved have been secured in as many different experiments. On the contrary, it is necessary, in order to determine exactly the relationships existing between interdependent linkage values, that all the data be obtained from the same experiment. This is because the precise value obtained for any given linkage is not only subject to the ordinary error of random sampling but may vary significantly in different experiments, in response to different environmental conditions, the age of parents, genetic factors, and the amount of discrepancy due to differential viability. Piling up enormous counts does not eliminate these sources of variation. Any slight aberration thus produced in the absolute value of one of the linkages (say AC) will then alter so materially its relative value, as compared with the other linkages (AB and BC), obtained in two different experiments, that the different values no longer fit into the linear system; they will not be expressible as any sort of function of the system. That is why Castle found that the *Drosophila* workers' own data gave the per cent. of crossing over between y(yellow) and w(white) as 1.1, between w and bi (bifid) as 5.3, and between y and bi as 5.5, a relationship quite at odds with their claims concerning linear linkage for short distances. Castle could have pointed out numerous similar "discrepancies," by similarly choosing to compare exactly (within, say, one unit of distance), the results of different experiments. In fact, had we been allowed to select the experiments for him, we could have chosen values such as the following: Sb (frequency between star and black) 39.3;

bp (frequency between black and purple) 5.9; Sp 0.4. If Castle will follow his usual procedure here, and represent these frequencies by proportionate distances in a model, he will disprove not only linear linkage but both Euclidean and non-Euclidean geometry and plain arithmetic. The trouble in the case just cited arises in the fact that the first two values are those obtained under ordinary circumstances whereas the third is a value obtained in the presence of the factor CHIL which decreases enormously the amount of crossing over. Clearly it will be unfair to expect a single map to represent all three values simultaneously. Nevertheless, similar although less exaggerated, disturbing influences may be, and frequently are, at work causing discrepancies between the results of "ordinary" experiments, so that it should be evident that the latter are not ordinarily fit to be subjected to the delicate comparison which is necessary for the purpose of determining the nature of the linkage system.

To some critics, it might at first sight appear inconsistent for the *Drosophila* workers to use the above argument against Castle's system, in view of the fact that these workers themselves also combine the results of different experiments in constructing their chromosome maps. The answer to this is that the variations in linkage between ordinary experiments are usually so small absolutely, that, if all the data for independent linkage values, like AB, BC, CD, etc.—are joined together and represented in one linear map, the latter will be accurate enough for the usual purpose of computing approximately the per cents of separation: the factors will appear in their correct order, and with approximately the correct distances between them. If, however, a study of the nature of the system of linkage is to be made, much more precise knowledge than this is required, for it is necessary to know exactly the relative strengths of interdependent linkages—like AB, BC and AC—as compared with one another. In such a case the small absolute deviations occurring in the different experiments become

large relative deviations of the linkages as compared with each other—this is particularly true the smaller the absolute per cents of separation are—and so a totally erroneous impression of the nature of the linkage system may be produced. The nature of the linkage system—whether it is linear and, if so, what function of a line is involved—can only be studied to the best advantage in experiments involving several factors at the same time, but if our judgments regarding it have already been arrived at, or verified, in this way, it is then quite legitimate to use this knowledge for other factors, and to join the results of different experiments involving them into one linear map.

TABLE II

SEPARATION FREQUENCIES BETWEEN EVERY TWO OF THE SIX SEX-LINKED FACTORS *y*, *bi*, *cl*, *v*, *s*, *B*, AS SHOWN IN A COUNT OF 712 FLIES FROM A CROSS IN WHICH TWELVE SEX-LINKED FACTORS WERE FOLLOWED SIMULTANEOUSLY. (MULLER)

Directness of the Linkage, According to the Linear Map	Factors Considered	Number of Separations	Per Cent. of Separations	Sum of Numbers of Separations of Each from an Intermediate Factor	Sum of Per Cents. of Separations of Each from an Intermediate Factor
"Primary" (for the experiment)	<i>y</i> and <i>bi</i>	39	5.5		
	<i>bi</i> and <i>cl</i>	53	7.4		
	<i>cl</i> and <i>v</i>	112	15.7		
	<i>v</i> and <i>s</i>	57	8.0		
	<i>s</i> and <i>B</i>	95	13.3		
Dependent on two "primaries"	<i>y</i> and <i>cl</i>	92	12.9	<i>y bi</i> + <i>bi cl</i> = 92	12.9
	<i>bi</i> and <i>v</i>	165	23.1	<i>bi cl</i> + <i>cl v</i> = 165	23.1
	<i>cl</i> and <i>s</i>	167	23.4	<i>cl v</i> + <i>v s</i> = 169	23.7
	<i>v</i> and <i>B</i>	152	21.3	<i>v s</i> + <i>s B</i> = 152	21.3
Dependent on three "primaries"	<i>y</i> and <i>v</i>	198	27.8	<i>y cl</i> + <i>cl v</i> = 204	28.6
	<i>bi</i> and <i>s</i>	216	30.3	<i>bi v</i> + <i>v s</i> = 222	31.2
	<i>cl</i> and <i>B</i>	240	33.7	<i>cl v</i> + <i>v B</i> = 264	37.0
Dependent on four "primaries"	<i>y</i> and <i>s</i>	247	34.7	<i>y cl</i> + <i>cl s</i> = 259	36.4
	<i>bi</i> and <i>B</i>	275	38.6	<i>bi v</i> + <i>v B</i> = 317	44.5
Dependent on five "primaries"	<i>y</i> and <i>B</i>	296	41.6	<i>y v</i> + <i>v B</i> = 350	49.2

In the experiments previously cited, the nature of the linkage in various sections of the chromosome has been

studied by following the inheritance of three factors in that region simultaneously. By a series of extensive counts of this sort the nature of the linkage in each individual section of the first chromosome has been studied, and found to be linear. The data in Table II are derived from an experiment which involves less extensive numbers than these, but illustrates to better advantage the linear behavior of all parts of the chromosome at once. These data are taken from Muller's cross of flies heterozygous for twelve mutant sex-linked factors. The results for six of these factors—those scattered most evenly along the chromosome—are shown in the table, which gives the number and per cent. of separation between every one of these factors and each of the other five. It will be seen that it happened that in this particular experiment, for all per cents of separation below 23, the per cent. of separation between any two factors was exactly equal to the sum of the per cents of separation of each from a third factor lying between them, whereas for factors less closely linked, the larger per cent. was less than the sum of the other two by an amount varying closely with the size of the large frequency itself. The data obtained in this same experiment for the three factors *y*, *w* and *bi* are given separately in Table III, in order that they may be compared to better advantage with the non-linear relation for these factors which Castle claims, as a result of his combination into one map of the results of separate experiments. Whereas Castle obtained a triangular figure to represent the three frequencies (*y w* 1.1, *w bi* 5.3, and *y bi* 5.5) it is seen that in this experiment, where all three were followed at the same time, an exactly linear relationship was obtained (*y w* 1.7, *w bi* 3.8, *y bi* 5.5). An experiment of Sturtevant's involving just these three factors is shown in the same table (III); here too the relations are entirely linear. In like manner the values obtained in the 12-factor experiment for the loci of *y w* and *A* are given in Table IV (*y w* 1.7, *w A* 1.4, *y A* 3.1) to be compared by the

"triangular" values (y w 1.1, w A 1.7, y A 2.0) claimed by Castle. The numbers in these experiments are quite sufficient to have revealed clearly any such triangular relationships as shown in the data chosen and figured by Castle.

TABLE III

SEPARATION FREQUENCIES OF y , w AND bl

*A. Data from the same experiment as that which furnished Table II.
(Muller)*

I. Non-separations	II. Separations of y from w and bl	III. Separations of bl from y and w	IV. Separations of w from y and bl	V. Total Files
673	12	27	0	712
Resultant Per Cent. of Separations Between y and w	Resultant Per Cent. of Separations Between w and bl	Resultant Per Cent. of Separations Between y and bl		
$\frac{(II+IV)}{V}$	$\frac{(III+IV)}{V}$	$\frac{(II+III)}{V}$		
1.7	3.8	5.5		

B. Data from a cross involving just these three factors. (Sturtevant)

I. Non-separations	II. Separations of y from w and bl	III. Separations of bl from y and w	IV. Separations of w from y and bl	V. Total Files
487	3	16	0	506
Resultant Per Cent. of Separations Between y and w	Resultant Per Cent. of Separations Between w and bl	Resultant Per Cent. of Separations Between y and bl		
$\frac{(II+IV)}{V}$	$\frac{(III+IV)}{V}$	$\frac{(II+III)}{V}$		
0.6	3.2	3.8		

TABLE IV

SEPARATION FREQUENCIES OF y , w AND A.

*(From the same experiment as that which furnished Tables II and IIIA.
Muller)*

I. Non-separations	II. Separations of y from w and A	III. Separations of A from y and w	IV. Separations of w from y and A	V. Total Files
690	12	10	0	712
Resultant Per Cent. of Separations Between y and w	Resultant Per Cent. of Separations Between w and A	Resultant Per Cent. of Separations Between y and A		
$\frac{(II+IV)}{V}$	$\frac{(III+IV)}{V}$	$\frac{(II+III)}{V}$		
1.7	1.4	3.1		

4. Although it has been shown that the linkage relations existing among the factors in any one experiment are functions of a linear series it might still be questioned whether there might not, after all, be some advantage in using Castle's system of graphic representa-

tion—whereby each separation frequency is supposed to be shown by an exactly proportionate distance on the figure, no matter how many dimensions may be required for this purpose. It will now be shown, however, that such a system of representation is impossible, quite aside from the fact that the models shown in Castle's papers are based upon data which can not legitimately be combined together. That is, no matter whether the data used are all derived from one experiment, or whether the results of different experiments are combined according to Castle's method, they could not be represented either in a three-dimensional or in any other geometrical figure, in such a way that all the distances would be proportional to the separation frequencies.

This may be illustrated by the data reported in Table II. It has been seen that the per cent. of separations between *y* and *cl* is exactly equal to the per cent. of separations between *y* and *bi* plus that between *bi* and *cl*. If then we represent these frequencies by actual distances, we must make the distance between points *y* and *cl* exactly equal to the distance between *y* and *bi* plus that between *bi* and *cl*. The only possible way to do this, on any kind of geometry—one-dimensional, three-dimensional or *n*-dimensional—it to put these three points in one straight line. In a similar manner we must place *bi* *cl* *v* in a straight line, and also *v* *s* *B*. *Cl* *v* and *s* are in almost a straight line, but there would have to be a slight bend at *v*, owing to the fact that *cl* *s* is very slightly shorter than *cl* *v* plus *v* *s* (on account of just one double crossover having occurred between them); this is correlated with the fact that *cl* *s* is a longer distance than the others considered. The figure so constructed, on the basis of Castle's own methods, is shown in Fig. 3; it is quite evident that this is the only figure which will represent directly (proportionately) the frequencies above considered. If, however, we now measure the distance on this figure between the extreme points, *y* and *B*, we find that it turns out to be 49.3, or very nearly the sum of

the intermediate distances (50.0), whereas the frequency of separation found between *y* and *B* in the actual experiment is 41.6. Similarly, the "model" shows too high a frequency for the other longer distances involved. (The long distances *y s* and *bi B* are 36.2 and 43.9 respectively on the model, but only 34.6 and 38.6 in the data; the moderately long distances *y v*, *bi s*, and *cl B*

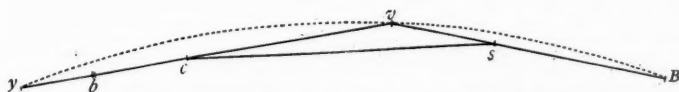


FIG. 3. Direct representation of the strongest and second strongest linkages in Table II. (*y bi*, *bi cl*, *cl v*, *v s*, *s B*, and *y cl*, *bi v*, *cb s*, *v B*, are each represented by a line of length proportionate to the respective frequency of separation.) The dotted curve shows the "average angular deviation" of the line of factors, according to this system.

are 28.6, 30.8, and 36.6, respectively, on the model, but 27.8, 30.3, and 33.7 in the data.) It would, on the other hand, have been possible to bring *y* and *B* close enough together in the diagram, and at the same time have adjacent factors the correct distance apart, by giving the line a curve, or bending it, as shown in Fig. 4. But if this is

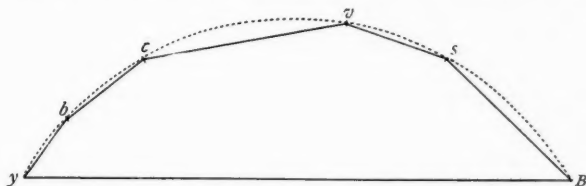


FIG. 4. Direct representation of the strongest and weakest linkages in Table II. (*y bi*, *bi cb*, *cb v*, *v s*, *s B*, and *y B* are each represented by a line of length proportionate to the respective frequency of separation.) The dotted curve shows the "average angular deviation" of the line of factors, according to this system.

done it is found that the distances of intermediate length (*y cl*, *bi v*, *cl s*, and *v B*) are not properly represented, all of them being relatively too short on the diagram. It would be unsafe to attribute these discrepancies, so uniform in direction, to the "errors of random sampling." The present experiment is cited, however, purely

as an illustration, to show what kind of discrepancies are meant. Discrepancies of exactly the same character and direction appear when the diagrams obtained by this method from experiments involving three points close together are compared with those from other experiments having three points far apart; that is, the former figures are repeatedly found to be nearer in form to a straight line than the latter; in such three-point experiments, moreover, highly extensive counts have been made, involving altogether (in the published experiments on the first chromosome), approximately a hundred thousand flies, and thousands of double cross overs.

5. The relation which has just been described, whereby the larger frequencies of separation are relatively smaller than could be directly represented in a curve constructed on the basis of the small frequencies, is due, according to the phraseology of the *Drosophila* workers, to the fact that the relative frequency of double crossing over ("coincidence") is so much larger for large frequencies than for small ones. Castle realizes to a certain extent the difficulty which this circumstance entails for his models, and he endeavors to meet it by means of the "subsidiary hypothesis" that the breaks in his models are more frequent in certain directions than in others. This assumption would, in some measure, explain away in a formal manner certain of the discrepancies (although cases of "triple crossing over" still remain an insurmountable obstacle), but the adopting of any such hypothesis really amounts to cutting away the ground from under the main theory of "proportionate representation," for the hypothesis involves an abandonment of the claim that the model represents each frequency by a proportionate distance between the nodes. For it is evident that if, in a given region, breaks in one direction are more frequent than in another, then points in this region which are an equal distance apart will be separated with different frequency according to the di-

rection of the line joining them. A given distance then no longer represents a given frequency.

6. It has been shown in the above two sections that a single figure will not represent accurately, by proportionate distances, the various linkage frequencies actually found in experiments involving many factors at once. If, on the other hand, it had been attempted to combine into one map the absolute frequencies obtained in a series of different experiments with two factors at a time, as Castle claims to do, the number and extent of discrepancies irreconcilable with any possible geometrical figure would have been much greater still. For, since the absolute frequencies found in different experiments necessarily have all sorts of irregular relationships to each other, it follows that it would be even less possible to show in one solid model separation frequencies which were obtained in this way. One such irreconcilable value has already been recognized by Castle—namely, the frequency *y* B. He is forced to represent this frequency by a curved wire in his model, because it is longer (being 47) than the longest distance possible (41) between these two points in any figure founded on a proportionate representation of the other frequencies. Of course, if distance on the model is to have any meaning, it cannot arbitrarily be represented along a straight line in some instances, and along lines having various degrees of curvature in other instances. In this one case, then, Castle is compelled to assume that the wrong value has been obtained, owing to experimental error, even though in all other cases he has assumed that it is quite legitimate to combine the results of different experiments. It would have been strange if, in making a model of this sort representing the separation frequencies of so many combinations of factors, Castle had not encountered more of these refractory cases. He does not mention any more, but it is noticeable that several other curved lines appear in his model. Moreover there is a conspicuous absence in the model of the factor lethal 2.

It would have been inconvenient to represent the observed linkage results for this factor by proportionate lines in the model, for, according to the results, lethal 2 would have to be placed at 9.6 from *w* and 17.7 from *v*, thus making the distance between *w* and *v* not greater than 27.3, whereas the established distance between *w* and *v* themselves is as much as 30.5; similarly, although only 9.6 from *w*, lethal 2 must be placed only 15.5 from *m*, although *w* and *m* are known to be at least 33.2 units from each other. Either the *wv* and *wm* lines would have to be considerably curved, therefore, or the lines between lethal 2 and the other factors would have to be *stretched* in some way—perhaps dotted lines would meet the difficulty!

Although any scheme of representing linkage results by exactly proportionate distances encounters the contradictions discussed above, it is noticeable that nearly all the most extreme departures from a plane curved figure (that figure which *comes nearest* to representing, by strictly *proportionate* distances, the ratios resulting from that type of linear linkage which actually exists) occur in the case of factors whose linkage ratios are distorted by differential viability or difficult classification. This is why the factors *A*, *fr*, *sh*, *cl*, *bi*, and the lethals stand out from the fairly regular curved line which Castle's models would otherwise conform to. The first two of the above factors are uncertain of classification, the others mentioned affect viability markedly. In the case of nearly all the remaining factors of the model, even though the results were taken from different experiments, it was nevertheless found, when the data were plotted, that they agreed pretty well with the expectations based on linear linkage. Moreover, the better the experimental conditions are in regard to viability, certainty of classification, and size of counts,—the more closely are these synthesized results of individual experiments found to coincide with the linear findings of experiments involving three or more points simulta-

neously. (It is for this reason that Sturtevant was first able to hit upon the general fact of linear linkage, on the basis of numerous careful experiments involving only two factors at a time.)

7. Owing to the inherent inconsistencies of the methods that were used to construct the solid models, it is to be expected that any predictions regarding separation frequencies which are deduced from them would be extremely unsafe. Castle states, however, that if any newly discovered gene has been located in the model, by obtaining its frequencies of separation from any three of the other genes contained therein, then the relation of the new gene "to all the others could be predicted by direct measurement from the model." In the case of two of the four predictions which Castle has made in this way, some evidence concerning the distance between the loci involved is already in existence.

One of the frequencies of separation in question is that between the loci of the recessive mutant factors glazed eye and rugose eye, in *Drosophila virilis*. Castle predicts, on the basis of his model, that the per cent. of crossing over between them should be found to be 4 or 5, or "probably a little greater." The work of Metz, and unpublished work of Weinstein, have shown, however, that hybrid females which carry both mutant factors exhibit the somatic character sterility possessed by the more extreme mutant type. When this dominance of an ordinarily recessive character in F_1 is taken together with the close similarity between the unusual effects produced by the two mutant factors (both produce a similar, peculiar effect on the eye, which is sex-limited, being more marked in the males), and with the fact that there is also a third mutant member of the series, with similar peculiar effects and similar linkage relations, it becomes highly probable that these factors are all allelomorphs. In that case they occupy "identical loci," and the frequency of separation between them must be 0. A direct determination of the per cent. of crossing over between

them is obviously impossible to obtain, on account of the sterility of the females carrying both factors.

Another prediction based on the solid models dealt with the frequency of separation between the factors hairy and magenta, of *Drosophila virilis*. It had been found by Metz that the per cent. of cross overs between hairy and forked was 3.1 and between forked and magenta 3.7; this would make the per cent. for hairy magenta 6.8 (or 0.6), if the factors were in a straight line. In the solid model, however, the arrangement of these factors, based on separate determinations of their frequencies of crossing over with distant loci,—is shown as triangular; and on the basis of this model Castle predicts that the frequency hairy-magenta will be found to be 4 or 5. The frequency has recently been determined by Weinstein, who has kindly consented to allow its use in this connection. He finds it to be 6.6.

It should be pointed out that Castle has endeavored to protect himself, in these predictions, by saying that they only hold, provided the relations given "have been determined with sufficient accuracy"!

8. One of Castle's specific objections to the linear maps, on which he lays much stress, is that on them the distances between the extreme factors is much more than 50 units, whereas factors which are linked must have a separation frequency of less than 50 per cent. It is only necessary to point out here that since, as we have seen, the linear maps, unlike the models, do not imply a proportionate relationship between the distances and the separation frequencies, these distances of over 50 do not connote separation frequencies of over 50 per cent. On account of the progressive reduction in separation frequency, due to double crossing over, that occurs with increasing distance, even distances of 100 or 110 in the second chromosome do not connote separation frequencies as high as 50 per cent. On the other hand, it should also be remarked that separation frequencies of over 50 per cent. would not be impossible *a priori*, as Castle

maintains; consequently any system of representing linkage which permitted or showed such values would not be *ipso facto* inconsistent. The mere fact that all factors hitherto worked with in a single chromosome have less than 50 per cent. of separation, and that those in different chromosomes have just 50 per cent., does not mean that factors can never be found which are so far apart, and which lie in such a rigid chromosome (little double crossing over) that they separate more often than they remain together at segregation. Whether this phenomenon should then be called linkage is but a question of words; the chromosomes themselves would have no regard for the 50.0 per cent. mark, or for the idiosyncrasies of our terminology.

The proof of the law of linear linkage, including all the main aspects of it which have been given above, has been stated on several previous occasions. It seems unfortunate that the argument has had to be repeated each time that a new "theory of crossing over" has arisen, for the discussion and data given in the original papers supply all the material necessary for a decision of the matter, at least so far as the germ plasm of *Drosophila* is concerned.

Before closing, it may be desirable to supplement these arguments for a mathematically linear mode of linkage, by a statement of the considerations which indicate that this mathematically linear linkage can have its basis only in a linear physical connection between the genes.

If the genes are not spatially arranged, or physically connected, in the same linear sequence as that in which they have been found to attract each other in linkage, then the forces of linkage attraction must be such as to "act at a distance." But, although acting at a distance, these linkage forces must nevertheless be extraordinarily specific—binding each gene directly to just two specific associate genes. Hence the forces could not be of an electrical nature, for, since there are only two kinds of electricity, electric forces could not be specific enough.

Similarly, the attractions could not be magnetic, nor could they be due to any kind of diffuse "physical" forces, such as those that emanate from centers of surface tension change or from centers of vibrational disturbances. Those who deny linear arrangement, while admitting the mathematically linear linkage results would therefore be driven to assume that the linkage attraction depended on the specific chemical nature of the genes, which, by virtue of their chemical composition, exerted a specific attraction at a distance, as the substances of adsorption compounds are sometimes supposed to do. But such a theory, as a method of accounting for linkage, becomes stretched to the breaking point when it is remembered that each gene must be assumed to have such an attraction for just two of the others, never more nor less, and that when this attraction is broken it is always exchanged for that of the allelomorph. Moreover, it would be exceedingly hard to reconcile this theory with the finding that changes in the nature of the genes—mutations—alter in no wise the sequence of their linkage attractions, and very rarely change even the strength of the linkages. And when we come to analyze the linkage relations in detail, and encounter the phenomenon of interference, we find relations that are entirely at variance with all our preconceptions concerning chemical attractions or chemical activity in general,—results that would force us to assume (1) that a breakage of the attraction between two genes leads to an increased attraction between the other genes and (2) that the amount of this increased attraction ("interference") depends solely on the directness of the connection ("distance") between these other genes and the one whose attraction was broken, being not at all influenced by the chemical nature of the broken attraction, or by the chemical nature of the other attractions themselves. The facts of "interference" or "coincidence" are thus diametrically opposed to a chemical view of linkage, although they, like all the other facts of linkage, are quite in accord with

ideas of a spatial, physical linear arrangement, their interpretation on the latter basis being natural and obvious.

The idea that the genes are bound together in line, in order of their linkage, by material, solid connections thus remains as the only interpretation which fits the genetic findings. In view of the additional fact that the chromosomes—themselves known to be specifically linked to the factor groups—can, at certain stages of their history, be seen to have the linear structure required, it would indeed be rash to adopt a different theory, without most cogent evidence of a startlingly new character.

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INFLUENCE OF THE MALE IN THE PRODUCTION OF HUMAN TWINS¹

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It is frequently pointed out that the father of twins can have little influence in determining their production; such production is purely a maternal quality, due to double ovulation. One possible way, however, in which the male may influence twin production is recognized, but this affects only 1-egg twins. Thus, if we assume that 1-egg twins are due to an early fission of the embryonic blastodisc, or if they are due to a secondary budding (following the method of the armadillo), then the sperm cell might carry the tendency to such fission or budding, as well as the egg cell. This possibility, however, does not help the statistical student of plural births, such as Weinberg, because he believes that the tendency to 1-egg twins is not inherited at all.

In the following study, there will be considered only the class of cases showing heredity most clearly, namely, those in which the principal fraternity under consideration has more than one pair of twins. Parents of such fraternities are spoken of in what follows as repeater fathers or mothers. Our query is then: "What is the relative importance in twinning of inheritance from the maternal and paternal sides, or what is the relative occurrence of twin labors in the close relatives of repeating mothers and of their husbands?"

To get an answer to this question, all available figures on twin repeaters were studied statistically. Of 355 labors occurring to the mothers of repeating mothers, 16 (4.5 per cent.) were twin labors. Of 289 labors occurring to the mothers of twin-repeating fathers, 12 (4.2 per cent.) were twin labors. These statistics thus indicate

¹ Read before the American Society of Naturalists, at Princeton, Dec. 30, 1919.

that the frequency of twins in the fraternities of fathers of twins is almost the same as that of twins in the fraternities of mothers of twins. Since the average proportion of labors which are twin labors is 1.1 per cent. for the population as a whole, we see that twins occur in the fraternities of repeating fathers as well as repeating mothers about four times as frequently as in the population as a whole.

To make use of more extended pedigrees, we may compare the tendency to have twin children on the part of *sisters* of the father and the mother of twin fraternities and on the part of *brothers* of the fathers and mothers of such fraternities. Then we obtain the results shown in the following table:

	Per. Cent. of Births that are Twin Births
Father's sisters' children	8.2
Mother's sisters' children	5.5
Father's brothers' children	6.5
Mother's brothers' children	4.5

From this table, most of the items of which were based upon ten or more twin labors, it appears that the sisters of twin-producing parents are more apt to have twins than the brothers of twin-producing parents; but the sisters of twin-producing fathers are more apt to have twins than the sisters of twin-producing mothers; also the brothers of twin-producing fathers are more apt to have twins than the brothers of twin-producing mothers. In all cases the proportion of twin births is very high, ranging from 4 to 7.5 times the average proportion of twin births in the whole population. These statistics then indicate that there is no important difference in the hereditary influence to twin production on the part of the father and the mother of offspring which include two or more sets of twins.

If, instead of considering the cases of twins in general, we pick out those of certain (or highly probable) *identical* twins, then we find, in 30 families with such twins, that the mothers came from fraternities in which (in 77 labors) there were 13 per cent. twin labors, and the fathers came from fraternities in which (in 38 labors) there were 13

per cent. twin labors. Here we see that there is an equality of the maternal and paternal influence and that there is a larger proportion of relatives of *identical-twin* producers who are twins than of producers of *twins in general*. Indeed, the occurrence of twin-offspring to the fraternities of the parents of identical-twin producers is proportionally 12 times as common as in the population at large.

Another way of testing the inheritableness of 1-egg twins is by getting the frequency-distribution of the sex of twins in repeater families—those in which the influence of heredity most clearly shows itself. In these, therefore, we expect nearly an equality of twins of similar sex and of dissimilar sex, provided 1-egg twins are not found in these clearly inheritable strains. In 160 pairs of twins in repeater families, of which the sex is given, there are 54 of unlike sex and 106 of like sex. Expectation in the case of binovular twins is that there will be an equality of like and unlike sexed twins. Any excess of like-sexed twins is to be ascribed to the occurrence of 1-egg twins. In the present case, there is an excess of 52 pairs of like-sexed twins out of 160 pairs of twins, which indicates that about 1 in 3 of the twins in repeater families are identical twins, and this agrees approximately with statistics obtained from the population as a whole. From this we reach the conclusion that the tendency to production of 1-egg twins is certainly not less common in the case of repeater families than in the case of families in which there is only a single pair of twins. The statement, therefore, that there is no hereditary influence to be detected in the case of 1-egg twins appears certainly to be incorrect. In fact, the presence of heredity is more striking than in the case of other twins and this leads us to conclude that the hereditary tendencies toward uniovular multiple production so obvious in armadillo (*Tatusia*) persists also in man.

Still another way of testing the relative influence of the mother and father in twin production is the comparison of cases in which the father of twins has married twice, and the mother of twins has married twice. An examination of our records showed 30 families where at least one

parent of twins has married twice. In 14 cases it was the father who married twice, in 15 cases the mother, and, in 1 case, both father and mother. In the 14 cases of father of twins who had married twice, there were twins by both marriages in 2 cases, or 14 per cent. of all such cases. In the 15 cases where the mothers of twins had married twice, there were twins by both marriages in 3 cases, or 21 per cent. of all such marriages. The numbers are small, but, so far as they go, in view of the average occurrence of twins in only about 2 per cent. of all marriages (and hence if chance only were at work in 4 per 10,000 of both pairs of double marriages), they indicate that the tendency to twin production is hereditary and also that not only the mothers but also the fathers have great influence in determining the production of twins.

All the foregoing statistics speak strongly for the view that the father has about as much influence in the production of twins as the mother. This result at first sight seems quite inexplicable and indeed to reduce the whole matter to an absurdity. If twin production is due simply to double ovulation, what can the father have to do with the result?

The present paper does not attempt to give a final answer to this inquiry. It attempts only to set forth a hypothesis which suggests a line of experimentation to answer the question more definitely. We have assumed that 2-egg twins are due to the simultaneous bursting of two Graafian follicles while single births result from the bursting of a single follicle. There is, however, a good deal of evidence that single births are not always the consequence of the bursting of a single follicle merely. There are indeed several other factors that determine a single birth, such as the failure of one of two simultaneously expelled eggs to be fertilized or the failure of one of two simultaneously expelled fertilized eggs to develop to maturity. That is, it may well be that two eggs are simultaneously ovulated much more frequently than at present recognized and that the comparative rarity of twin-births is due either (1) to a failure of fertilization of one egg or (2) to a failure of development of one egg.

The conviction that not all eggs that are ovulated are fertilized is borne upon one who compares the number of corpora lutea in mammals that have large litters and the number of embryos that one finds in the uterus. I have recently made a number of counts in this respect in the case of sows and give below results in tabular form:

Observation Number	Number of Recent Corpora Lutea	Number of Embryos Found	Average Length of Embryos
1	3	3	15 cm.
2	6	3	10 cm.
7	8	7	6.5 cm.
12	9	2	2.5 cm.
13	8	7	
	34	22	

Thus from 34 corpora lutea, or 34 eggs expelled, only 22 embryos were found, counting only those which had reached a length of 2 cm., at which stage the chorion is already so large that it seems improbable that it should have been overlooked.

There is some reason for thinking that in humans also a certain proportion of the eggs ovulated fail of fertilization even in families in which there is no prudential restriction—in which the size of the families indicates a probability that nearly the maximum number of eggs became fertilized. Conclusions are fortified by the examination of a good genealogy including families of children born in the latter half of the eighteenth and the early part of the nineteenth centuries. Thus in a genealogy of the Gorton family, seventh generation, the intervals in round years between births in various fraternities (all related as cousins, are:

13 children—3, 1, 1, 5, 1, 2, 2, 2, 2, 1, 5; all born 1795–1821.

10 children—2, 2, 2, 2, 3, 2, 2, 2. In this case there is no unexpectedly large interval.

11 children—2, 2, 2, 2, 2, 2, 2, 1, 5.

6 children—4, 2, 4, 3, 4; all born between 1792–1809.

8 children—2, 2, 2, 2, 5, 2; all born between 1796–1813.

13 children—1, 2, 1, 2, 2, 3, 3, 3, 2, 2, 4. In this case also

there seems to be no failure of fertilization, except at the end of the series.

9 children—4, 2, 2, 2, 2, 2, 2.

10 children—3, 1, 4, 2, 1, 2, 2, 2, 2.

9 children—1, 2, 3, 4, 3, 2, 2, 3; born between 1825–1845.

One gets the impression that the normal interval between births, assuming all eggs to be fertilized, is about 2 years. The frequent intervals of 3, 4, 5 and even more years probably correspond to failure to fertilize, although they may be due to miscarriages or even in some cases to prolonged absence of the husband. In view of the fact, however, that we have to do here with a prevailingly rural population, chiefly farmers and millers in central New York State, the latter contingency is improbable.

The failure of fertilized eggs to complete their development is a real factor that must be taken into account. Attention has been called to the importance of this factor by John Hammond (*Journal of Agricultural Science*, VI, 1914) who has studied fetuses of rabbits and pigs and finds among them many degenerating individuals. Thus the number of degenerating fetuses in a large number of uterine horns examined varied from 0 to 19 per cent. I can confirm these results by observation made upon the uterus of a sow (No. 3) in which there were 2 corpora lutea in the left ovary and 5 in the right. In the left horn of the uterus there was a well-developed embryo 8 mm. long and one, evidently blighted, of 4 mm. The outlines of the latter embryo were highly abnormal and shrunken. The right horn of the uterus contained one embryo, 25 mm. long, a second 9 mm. long, and a third 6 mm. long. Thus with 7 corpora lutea in the ovaries, there were only 5 embryos found, of which one was completely blighted, another at 6 mm. length would probably soon have ceased development and two others at 8 and 9 mm. were far behind the best developed embryo, already 25 mm. long.

Work on yellow mice, of which the yellow \times yellow matings give rise to 25 p. c. atretic embryos, and the far more extensive experience of Morgan with lethal factors in *Drosophila*, indicate that failure of development is a far

more common phenomenon than hitherto appreciated. Lethal factors, it may be pointed out, are a probable solution of one of the mysteries of gynecology; namely, that a woman who is sterile with one husband is often fertile with another, even when examination has shown no defect in the spermatozoa. Similarly a husband may have no children by one wife, but one or more by a second marriage. Parallel phenomena are common in dairy cattle. We conclude then that lethal factors are probably widespread phenomena even in human germ cells, and account for a certain proportion of long intervals between births, of early miscarriages, and of sterile unions.

The application of the foregoing two principles of failure of fertilization and failure of development to the question of the rôle of the male in twin production is now fairly obvious. More eggs are laid, even without prudential restraint, than come to development, and this is true not only of eggs laid successively but of eggs laid simultaneously; that is, twins that are born are the residuum of a greater number of twins that are started in their development and of a still greater number of pairs of eggs simultaneously ovulated.

The literature of gynecology is indeed full of cases of blighted twins. In a fairly large proportion of all twin births, one of the twins has remained at a stage of development of the third, fourth, or even earlier month. The fetus is often found compressed and flattened; the name is given of papyraceus twin. The number of blighted twins which have been referred to in the literature amounts to several score, but naturally is a very small proportion of the whole. The vast majority of blighted twins are simply lost unnoted with the afterbirth. A record is made only of the larger blighted fetuses; the others are entirely overlooked, since search is rarely made for undeveloped embryos in the afterbirth, and the birth is consequently regarded as a single one. We must believe that a certain proportion, perhaps a large proportion, of the fraternities which show two or three twin labors interspersed with single labors are those in which pairs of eggs

have been ovulated in each case, but one of the pair has failed to develop, either through failure of fertilization or early blighting.

Now the lethal factors show their influence first in certain combinations, just as in the matings of yellow \times yellow mice. The $\frac{1}{4}$ of the embryos which die are those which are derived from germ cells containing the genes for yellow, whereas the other $\frac{3}{4}$ may develop fully. So we conclude that among humans the cases of twin-repeating fraternities are those in which there are no or few lethal factors in the germ cells, so that there is a maximum fertilization and development of the eggs laid.¹ In the case of families comprising only one pair of twins, combined with a number of single births, it is probable that in other cases there had been a double ovulation but one of the pair had failed to develop. The additional fact to be taken into account is that twins are found in a higher ratio in large families than in small ones. Large families, however, connote high fertility of the male as well as the female. From all these facts we reach the conclusion that families which readily produce twins do so not only because in the mother the eggs were laid in pairs, but also because in the father the sperm is active, abundant and without lethal factors, so that the number of eggs fertilized and brought to full term approaches a maximum. To repeat, such fathers, experience indicates, belong to strains which are exceptionally fertile and in which twins are repeatedly produced both along male and female lines. Thus it comes about that the fathers of twins are about as apt to belong to twin-producing strains as mothers of twins and that twinning depends on constitutional—hereditary—factors on both sides of the house.

¹ F. H. A. Marshall (1910), "Physiology of Reproduction," p. 618, recognizes that certain abortions in sheep "may be due to a want of vitality on the part of the developing embryo." Similarly gynecologists recognize that a part of the 10 per cent. of barren marriages, and many of the early miscarriages, have no explanation in pathology, but apparently only in physiology.

INHERITANCE OF CONGENITAL PALSY IN GUINEA-PIGS¹

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INTRODUCTORY

IN 1914 a litter of two guinea-pigs was born in our laboratory, one of which differed from the other in that it appeared to lack nervous control. When this individual was placed on its feet, attempts on its part to walk resulted in spasmodic stiffening of the legs, causing it to fall over on its side, where it lay helpless and unable to get up. Although the animal appeared otherwise in good physical condition, it was thought at the time that the trouble might be due to temporary nutritional disturbance, and attempts were accordingly made to feed it by hand,

¹ Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 18. Published with the approval of the Director of the Station.

until it should be able to nurse. The effort was, however, unavailing, the subject gradually becoming weaker and the symptoms more pronounced, until death ensued in a few days. A second litter was produced by this pair on May 19, 1914. This consisted of three young, which appeared in all respects normal. On August 27, however, another litter of two was produced and one of these was like the abnormal individual described above. This gave rise to the suspicion that the defect might be due to some hereditary cause and consequently the mating of the same parents was continued, with the following results: November 6, 1914, one defective offspring; March 24, 1915, three offspring, 2 being normal and one born dead; and June 20, 1915, 2 normal, 1 defective and 1 born dead. Not counting the two born dead, since their condition with respect to normal reactions could not be determined, this pair then produced a total of 13 young, of which 9 were normal and 4 defective. These results not only strengthened the presumption that we had to deal with a heritable condition, but were so close to a three-to-one ratio as to suggest that it might be a simple Mendelian recessive. It may be stated at this point that further extensive experiments have proven conclusively the correctness of both of these presumptions.

Full discussion of the symptoms and of related conditions in man and other animals will be reserved until the experimental results have been presented. It is sufficient to say here that the defective condition is always clearly marked and easily recognizable, and that in no case have there been doubtful intermediates. Furthermore, such efforts as have been made as yet to rear the defective offspring have been uniformly unsuccessful; these individuals always die within a short time, usually within two weeks of birth.

ORIGIN OF PALSIED STOCK

Later in 1915 palsied offspring were produced by other parents and studies of the pedigrees have shown that such individuals have appeared in three distinct lines

which are unrelated so far as the pedigrees show back to our original stock. This stock came from two sources, a few animals received from Professor Castle, of Harvard University, and somewhat less than a dozen young animals supplied us by our veterinary department, but obtained from a dealer. This stock had multiplied to about forty individuals at the time records were begun on it. The pedigrees show that in all probability there was only one individual, a male, in the Castle stock which might have brought in the palsy character, and since Professor Castle informs us that he has never noticed it in his animals, this individual may with considerable certainty be ruled out as the source of the defect in our experiments. The young animals received from the dealer were all of about the same age and were white spotted, very similar in appearance, which suggests that they may have been related. We are accordingly led to conclude that the character was introduced with this stock and that in all probability it may have traced back to one, or at least only a few, heterozygous animals, and that, furthermore, if there were more than one they were probably related.

INHERITANCE OF THE PALSY CHARACTER

The factor for normality appears to be completely dominant and we have found it impossible to distinguish animals carrying the defective trait from those which do not on the basis of observable behavior or any other characters. The only method of separating the two classes is therefore by breeding tests. Owing to the fact that the affected (recessive) individuals always die, it has been necessary to conduct the experimental tests by the round-about method of always mating animals to be tested to others known to be heterozygous. If the individual being tested was a homozygous normal no defective offspring would be produced by such a mating, whereas if it was heterozygous they should appear in the usual ratio for Mendelian recessives. We have therefore conducted extensive experiments to determine, (1) the ratio of palsied

offspring when two heterozygotes are mated, (2) the proportion of homozygous to heterozygous individuals among the normal offspring of such matings, and (3) the ratio of homozygous to heterozygous offspring when homozygote was mated to a heterozygote.

Since for practical reasons the number of offspring which can be produced from any particular pair of parents is limited, it became necessary to set a definite arbitrary number which should be taken as the minimum to indicate a fair probability that the animal being tested was homozygous for normality if no recessive young were born. Five was chosen as this minimum, but in every instance larger numbers were obtained where possible. All cases in which less than five normal offspring were obtained without the appearance of a recessive are discarded from the calculations. Furthermore, only six of the thirty individuals rated as homozygous normals on the basis of their breeding behavior had so few as five offspring, and in most cases the number was considerably larger, as is shown in Table I.

TABLE I

ANIMALS RATED AS HOMOZYGOUS NORMAL AND THE NUMBER OF EXCLUSIVELY
NORMAL OFFSPRING ON WHICH THE RATING WAS BASED

No. of Animals Tested	No. of Normal Offspring Produced by Each	Total Offspring
62	5	30
32	6	18
12	7	7
1	8	8
1	9	9
4	10	40
6	11	66
1	12	12
1	13	13
2	14	28
1	15	15
1	23	23
1	24	24
1	26	26
Total 30	—	319

² Eight of these ten animals died before more offspring could be obtained, one was discarded because of being a poor breeder, and one for some unassigned cause.

Further evidence that this test is fairly reliable is furnished by the fact that in matings of heterozygote to heterozygote affected offspring appeared in litters before five normal young had been born in 84 per cent. of the cases, while in 22 of the 32 matings the recessive appeared in the first litter. The complete data are given in Table II.

TABLE II

Number of Matings	Number of Normal Offspring Produced before Litter Containing Recessive
22	0
3	1
2	4
3	5
2	8
32	—

For present purposes we have adopted the symbol *N* to represent a factor for normality; the recessive, palsied animal is therefore *nn*.

1. *Ratio of Palsied Offspring when two Heterozygotes are mated.*—As there appears to be no need of presenting the detailed data of individual matings, the combined results of mating heterozygous animals together are given in the left hand side of Table III. Of the total number

TABLE III

MATINGS *Nn* × *Nn*

	Offspring			Tested Normal Offspring	
	<i>N</i>	<i>nn</i>	Born Dead	<i>NN</i>	<i>Nn</i>
Observed	183	63	36	7	15
Expected	184.5	61.5	—	7.3	14.6

of offspring alive when born (that is, when found) 183 were normal and 63 palsied, an almost exact three-to-one ratio. We, therefore, feel safe in our assumption that the palsied condition is based on a single unit factor difference. The question might be raised as to whether the rather large number of offspring "born dead" might not represent a disproportionate number of palsied young. This does not, however, seem probable for a number of

reasons. In the first place, the palsied animals appear as strong and vigorous when born, and in all respects except for the nervous condition as fully developed as the normal young. This is further borne out by the weights, the weight of the palsied young being as great, indeed averaging slightly more at birth than that of the normals. The weights at birth of the living young from $Nn \times Nn$ matings are shown in Table IV, from which it will be seen

TABLE IV
WEIGHTS AT BIRTH OF LIVE OFFSPRING PRODUCED BY MATINGS $Nn \times Nn$

	Number Weighed	Average Weight in Grams	Number Not Weighed	Total Individuals
Normal males	91	88.61	4	95
Normal females	79	88.68	9	88
Normal males and females combined ..	170	88.65	13	183
Palsied males	32	92.21	0	32
Palsied females	29	87.96	2	31
Palsied males and females combined ..	61	90.19	2	63

that 170 normal offspring averaged 88.65 grams, whereas 61 palsied young average 90.19 grams. The slightly greater weight of the latter is probably not significant. These facts seem to indicate strongly that the congenital death rate was not differential with respect to palsy.

2. *Proportion of Homozygous to Heterozygous Individuals among the Normal Offspring of $Nn \times Nn$ Matings.*—Further proof that we were dealing with a single factor difference was provided by tests of the normal offspring from matings of heterozygote to heterozygote. These should, of course, consist of two heterozygous individuals to each extracted homozygous dominant, which should breed as free from the defect as any animals from non-palsy stock. As shown in the right-hand half of Table III, 22 of the 183 normal individuals were tested, of which 7 proved to be NN and 15 Nn , the theoretical expectations being 7.3 and 14.6, respectively.

3. *Ratio of Homozygous to Heterozygous Offspring from Mating $NN \times Nn$.*—One other type of test was

made, namely, of the normal offspring resulting from the mating of homozygous to heterozygous individuals. The expectation in this case is equality of the classes, and the

TABLE V
MATINGS $NN \times Nn$

	Offspring			Tested Offspring	
	N	nn	Born Dead	NN	Nn
Observed	319	..	36	14	11
Expected	319	0	..	12.5	12.5

actual numbers found in the 25 tests made were 14 NN and 11 Nn , the expectation in this case being 12.5 in each class (see Table V).

Additional evidence that the extracted homozygous normals are free from palsy "taint" is furnished by three matings of such animals together, from which 31 living offspring have been obtained, all normal.

The foregoing data would appear to be sufficient in number and in closeness of ratios to demonstrate conclusively that congenital palsy in guinea-pigs is inherited in simple Mendelian fashion and depends on a single unit difference, the normal condition being completely dominant to the heterozygote.

SYMPTOMS

A brief description of the typical symptoms has already been given, but for comparison with the same or similar conditions which may be observed by others, it seems desirable to describe the symptoms of the palsy as it occurs in our stock in somewhat greater detail.³

A word ought perhaps to be said at this point about the use of the term congenital palsy. The congenital part is evident enough and needs no explanation further than to point out that we use it in the sense of being present at

³ We wish to express our appreciation of valuable advice and assistance rendered us by Dr. W. J. Meek in connection with this and the following sections of this paper.

time of birth rather than of being contracted at time of birth, which is the connotation sometimes implied in relation to certain infectious diseases. The word palsy is used in the general sense to indicate the broad similarity of the condition in the guinea-pigs to trembling palsy in man. The term is intended to be a neutral one with no implications as to the ultimate cause of the disturbance. The condition perhaps in some ways more closely resembles tetany as manifested in mammals below man, but this term has been avoided as having possibly too specific an implication.

There is considerable variation in the degree to which different individuals are affected. In most cases the victim when discovered shortly after birth is lying on its side slowly moving the legs, twisting the body and lifting the head as if in a vain endeavor to get on its feet. The movement of the fore part of the body, head and forelegs is much more pronounced than that of the hind quarters and hind legs. Some individuals if placed gently on their feet are able to stand, though usually in a strained tense attitude. The difference between this and the normal position may be observed in Fig. 1, ♀ 1089.1 being a palsied individual, while the others are its normal brother and sisters. The photograph is taken from directly above. The affected individual has the feet somewhat spread and the body slightly contorted, while the others are in natural easy attitudes.

If left quietly to itself after being placed on its feet the animal usually stands unsteadily for a few moments and then when it starts to walk falls on its side, with characteristic movements of the legs to be described presently. Some animals are so little affected at birth that they are able with effort to gain their feet themselves, and to walk about in a clumsy, jerky, paralytic fashion. They experience the most difficulty in the control of the hind legs, which appear to be in a hypertonic state and are commonly moved more in a hopping fashion than in steps. A rough classification of 51 palsied animals soon after birth gives the following: 14 unable to rise and unable to stand

when placed on their feet; 18 able to stand but unable to walk; 5 able to walk when placed on their feet but unable to arise unaided; and 14 able to get up and to walk. It should be recalled that in all cases the symptoms grow progressively worse, leading to the most severe conditions, and to death in a week or two at most.

Breathing appears to be normal, as is also control of the muscles of the jaws and throat, for the less affected animals sometimes eat solid food, and those that are able to walk may suckle the mothers. Such individuals increase in weight for a time as rapidly as normal young, but with the progress of the disease they become unable to obtain nourishment, and consequently decline. We are unable to state at present whether death is attributable finally to starvation, or whether it is a direct sequel of the disease.

The most striking phenomenon in connection with the disease is the reaction to stimuli, particularly to auditory stimuli. This may be best observed in animals that can stand when placed on their feet but are able to walk only with great difficulty, if at all. If such an animal is placed on its feet and a sharp sound is then made, such as clapping the hands, snapping the fingers, or squeaking with the lips, the reaction is definite and immediate—the subject jumps upward and forward, due to a sudden stiffening, particularly of the hind legs, then falls on its side, the whole body shaking to some extent, but the legs exhibiting strong clonic spasms. To the same stimulus normal individuals give merely a slight start, and then sit unconcernedly as before. This result is clearly shown in Fig. 2, which depicts the same litter as Fig. 1, but following a stimulus which has thrown the affected individual into a spasm as described. Fig. 3 is a short time-exposure of an animal in a spasm lying on its side. The photograph shows clearly the movement of the feet.

Visual stimuli have relatively little effect in producing the above-mentioned reaction. Even if the hand is brought rapidly down to near the animal's eyes it seldom responds. The same is true for mechanical stimuli, the reaction occurring only if the stimulation is severe. Af-



1 ♂ 1089.3 ♀ 1089.4 ♀ 1089.1 ♀ 1089.2
 NN Nn nn Nn



2 1089.3 1089.4 1089.1 1089.2



3 758.1

INHERITANCE OF CONGENITAL PALSY IN GUINEA-PIGS.

affected animals which are fairly able to walk may not fall over even as a result of an auditory stimulus. They give a jump, much more pronounced than the start of normal individuals, but manage to stay on their feet. Furthermore, even the more affected ones become less sensitive to repeated stimulation, and may after several reactions fail to respond sufficiently to make them lose their balance.

In the more severe stages the reaction appears to simulate intentional tremor, in that it follows attempts at voluntary movements of the hind legs. In less severe cases the animal can use the legs if free from other nervous excitation. It would appear therefore that the condition is induced by sudden nervous excitement, the degree of the stimulus necessary to cause complete lack of muscular control depending on the stage of progress of the affection.

The severe spasms commonly last but a few moments. If a guinea-pig stiffened out in one of the spasms is taken in the hand it can soon be felt to relax, following which it either lies quiet or makes slow movements of the head and to some extent of the legs as previously described.

ETIOLOGY

A number of possibilities suggest themselves as causes of the disease described in this paper, and these will be discussed in order.

1. As mentioned in the following section, digestive disturbances may cause in sheep a condition very similar in many of its symptoms to the spasm of our guinea-pigs. Is it not possible that these were originally induced by some similar cause? It is true that at times, especially in the early part of the work, we have had some trouble from improper feeding, notably when we attempted to substitute sugar beets for carrots and cabbage. While, however, inadequate diet may cause scurvy and other effects, we have no reason to believe that it ever produces a condition which could be mistaken for the congenital palsy. Furthermore, palsy never occurs in the descend-

ants of two homozygous normal individuals, even though their feeding and care is in all respects similar to that of the others. In other words, the disease has behaved strictly in accord with the known principles of heredity since it has been under observation, and we have every reason to believe that it has not appeared spontaneously during that time. This would mean then that if the disease was due to nutritive conditions, it must have had its inception in the stock before we received it, or within a very short time thereafter at latest.

The evidence that heritable defects of this sort may be induced by external conditions is very meager at best, Stockard has produced somewhat similar nervous defects in guinea-pigs by the administration of alcohol, but while he claims that these are heritable, he has not, so far as we are aware, shown that any of them are inherited in strict Mendelian fashion as is the defect with which we are concerned. We are therefore led to believe that this character in our stock has not been induced by nutritional or other environmental causes, but that it is due to a factor mutation similar to those which have been studied so thoroughly in domesticated and experimental animals and plants in recent years, and for which there is at present no assignable cause.

2. It might perhaps be assumed that the palsied individuals are due to unfavorable uterine conditions and consequent abnormal fetal development. The occurrence of "runts" in swine and in other animals which produce large litters show that the uterine conditions are not the same for all the individuals in a litter. Some doubtless have a poorer maternal blood supply than others, or they may be crowded, or twisted into a position unfavorable for growth. That these are not factors in the present case seems demonstrated, however, by the fact that the palsied animals are on the average fully as large and well developed at birth as their litter mates (see Table IV).

3. The necessity of inbreeding the original stock in order that the recessive palsy condition should appear is apparent; but it might be maintained that this inbreeding

in itself was perhaps the cause of the disease. All modern studies on inbreeding, however, seem to strengthen the conclusion that while inbreeding may, by the "concentration" or production of unfavorable character combinations, and particularly by the loss of important physiological factors which are necessary to the well being of the individual, result in lowered vitality and in the appearance of various defects, it is nevertheless a means rather than a cause of bringing these into expression.

Further evidence that the palsy was not produced by the inbreeding as such is furnished by the fact that the lines in which it appeared were no more inbred than many other lines that have been carried on in the laboratory in connection with other problems, but in which no tendency to such a defect has manifested itself. Our whole stock, in fact, of some 2,200 litters and over 5,000 offspring has all descended from not more than 50 original animals, and as has already been stated, there is reason to believe that some of these were related. In order to show the intensity of the inbreeding in some cases, it may be mentioned that in one experiment a male was bred back to his daughters for four successive generations, and with no apparent ill effects. Inbreeding as a predisposing cause may therefore be ruled out.

4. The spasms which form the characteristic reaction of the palsied animals are clearly due to lack of nervous control, especially when voluntary movements of the legs are attempted, and under excitement. It may therefore be that there is some heritable defect of the central nervous system. Examinations which have been made for us by Dr. C. H. Bunting have, however, shown no lesions of the nervous system to which these effects could be attributed.

5. Disturbances of some of the glands which supply internal secretions are known to produce nervous irritability and conditions of spasm and tetany. Particularly is this true of the parathyroid, and some of the symptoms accompanying disturbances of this gland resemble to a certain extent, at least superficially, the con-

ditions in the guinea-pigs. Dr. Bunting is making a study of this phase of the question, and reports that "thus far the only anatomical difference between affected and normal animals of the same litter, that has been noted, has been a definite hypoplasia of the parathyroid tissue in the abnormal animals." He will, however, report more fully later.

For the present, therefore, we must be content with the statement that the congenital palsy is due to a factor mutation, the cause of which is unknown; nor do we understand what its action is on the animal organism to produce the nervous symptoms described.

DISCUSSION

A number of nervous defects are known in man and the lower animals which have certain points of resemblance to congenital palsy as it occurs in our guinea-pigs, but we have been unable to find any condition which agrees closely enough in details so that the two could be considered identical. A list of some of these follows, with brief mention of resemblances and points of difference.

Pigeon.—*Tumbling* in pigeons appears to be due to lack of nervous control of the muscles and associated to some extent with certain voluntary efforts. This is especially noticeable in Parlor Tumblers, which turn back somersaults when they attempt to fly. The condition is greatly exaggerated by excitement. Further similarities are that the tendency to tumble increases with age, to a certain point at least, and that it behaves in a general way as a recessive to normal flight, though the crossbreds are usually intermediate and there appears to be no sharp segregation in F_2 . Tumbling, unlike congenital palsy, does not seem to affect the legs particularly, and does not interfere with normal life processes sufficiently to be lethal if the birds are given adequate protection and care.

The condition described by Riddle (1918) as *ataxia* in pigeons would appear to correspond very closely in symptoms to the more pronounced cases in Parlor Tumblers.

There would appear to be also some resemblance to the tumbling and shaking of the Fantail (French *Trembleur*). Riddle states that the "character is, with some irregularities, a Mendelian recessive." His inference that it may have been produced by "reproductive overwork" seems inconclusive.

In connection with experiments on the homing ability of pigeons Hurst (1913) speaks of obtaining "*feeble-minded*" birds, as follows:

Results show that incompetent or feeble-minded pigeons may be bred from competent or intelligent parents, and it is interesting to find that feeble-mindedness behaves as a recessive character in birds as well as in man.

Fortunately, or unfortunately, it is much more difficult to get offspring from the feeble-minded in Pigeons than in Man.

Mouse and Rat.—The well-known "waltzing" of the waltzing mouse is probably of the nature of a nervous disorder, either directly or indirectly. It is a simple Mendelian recessive.

Bonhote (1912) at a meeting of the Zoological Society of London "exhibited living specimens of rats (*Mus rattus*) which he had bred in the course of his experiments, and which showed the 'waltzing' character well known in a variety of the domestic mouse, but which had not hitherto been recorded in rats."

Rabbit.—We have in our possession a rabbit which is now several years old, and which has since it was young exhibited characteristic circus movements, or "waltzing," very similar to the activities of the waltzing mouse. This character appeared sporadically and we have been unable to find that it is heritable, even though we have repeatedly bred this male's daughters to their own brothers and back to him. He appears normal in other respects except that one eye seems somewhat distorted, which may have something to do with his behavior. This case differs from the waltzing mouse in that it is probably not heritable, and certainly is not a simple Mendelian recessive.

Guinea-Pig.—Some of the various defects in guinea-

pigs described by Stockard and ascribed to the inherited effects of alcohol treatment of the original parents, have symptoms somewhat resembling those of congenital palsy. His descriptions of the symptoms and behavior of his animals are, unfortunately, inadequate for detailed comparison of our cases with his. One point seems certain, however, namely, that while the symptoms exhibited by our animals are relatively constant, he has obtained in his affected lines a great variety of nervous defects and anatomical abnormalities, all of which he attributes to degeneration caused by the alcohol. To mention those relating to nervous disorders, he speaks of the defective animals as being "very shy and excitable" (1912, p. 22), and says further: "it is a point of some interest that all of the young animals that died showed various nervous disturbances, having epileptic-like seizures, and in every case died in a state of convulsion." Again (1913, p. 663) he speaks of an animal which died when one day old, "having been in a constant tremor since its birth; another lived for nine days *but whenever it attempted to walk it was seized with spasmodic contractions*; the third specimen exhibited the same nervous manifestation and was completely eyeless." In a later paper (1916, p. 15) he says that *paralysis agitans* is very common among the F_1 , F_2 , and F_3 animals, apparently applying this term to some of the symptoms mentioned in earlier papers, and adds that "paralyzed limbs are often observed, the animals being unable to stand or walk."

While some of the above symptoms approximate those of congenital palsy, they seem to partake for the most part either of a general nervous irritability or else of a definite paralysis. Furthermore, while Stockard bases his conclusion that the general defect that produces these various conditions is hereditary on the fact that they continue to appear in his treated lines, but not in the parallel control lines, he has not so far as we are aware, found any tendency for the condition to be inherited in any definite manner or proportions conformable with Mendelian rules.

It is not clear, however, that he has made any systematic matings in an endeavor to ascertain this point.

While we do not mean to imply that in Stockard's experiments the initiation of the various defects and abnormalities he describes may not have been due to the alcohol treatment, it is nevertheless of interest to note that these same defects and abnormalities appear from time to time in our normal stock. We can, in fact, match his conditions—almost case for case for all that he has described—with offspring of our stock that has had the best care we could give it. That our stock is as a whole in no way degenerate is indicated by its prolificacy (average size of 863 litters = 2.71), its low mortality rate, and the fact that our animals are if anything above the general run of guinea-pig stock, as attested by reports from the various hygienic and other laboratories to which our surplus has gone. The point of special interest is that these various abnormalities are entirely independent of the congenital palsy, for they appear no more frequently in the "palsy" stock than elsewhere.

Goat.—Hooper (1916) has described a case in goats which has some strong points of resemblance to the behavior in the guinea-pigs, although in the former the conditions are not so severe as to cause the death of the animals. He says:

There is a peculiar breed of goats raised in central and eastern Tennessee. When suddenly frightened the hind legs become stiff and the animal jumps along until it recovers and trots off normally or if greatly frightened the front legs become stiff also and the goat falls to the ground in a rigid condition. They have received the name of "stiff-legged" or "sensitive" goats.

Experiments were to be begun on the inheritance of the character, but results have not to our knowledge been reported.

Sheep.—A condition in sheep with symptoms somewhat resembling those in "palsied" guinea-pigs and even more the goats just mentioned is described by Jones and Arnold (1917). Affected animals are able to walk, but

when excited they run in a stumbling fashion and finally the legs stiffen out and the animal falls on its side. This affection is, however, not heritable, but has been demonstrated by these investigators to be due to nutritional disturbance, caused by a diet consisting too largely of pampas grass.

When there is a liberal amount of grass the actual number of cases is small. After a long continued drought when the fine grass supply is short, the number of sick animals is large. The mortality varies considerably, young sheep seeming to suffer most.

Man.—Among the numerous confusing and complex nervous disorders in man there are several with certain similarities to congenital palsy of the guinea-pigs. We have not attempted an exhaustive survey of this field, but list a few of them with remarks on resemblances or dissimilarities. In some cases it is difficult to tell whether the descriptions refer to the same or different affections, the synonymy not being clear. The comparison with congenital palsy is also often uncertain owing to the indefiniteness of the descriptions of symptoms. No attempt at completeness has been made in the matter of references, citations being added merely for giving authority for the statements made.

Feeble-mindedness (Davenport, 1911), *epilepsy* (Davenport, 1911; Davenport and Weeks, 1911; Weeks, 1915) and some forms of *insanity* (Davenport, 1911) resemble congenital palsy in being definitely recessive in inheritance, but show no close similarities in other symptoms.

SIMILARITIES	DIFFERENCES
<i>Paralysis agitans</i> (Parkinson's disease). (Curschmann, 1915.)	
Tremor of muscles.	Appears late in life.
Progresses in severity with course of disease.	Constant trembling. More often in male sex.
<i>Habitual tremor.</i> (Curschmann, 1915; Dana, 1887.)	
Occurs early in life.	Not congenital.
Subsides when patient is at rest.	Affects mostly hands and head.
Increased by voluntary movements and excitement.	Shallow oscillations. May disappear.
Tendency to be hereditary? (Occurs mostly in neuropathically inclined individuals.)	

Familial tremor. (Curschmann, 1915.)

Hereditary.	Not congenital.
Usually appears in youth.	Sometimes improvement.
Diminishes at rest.	
Affects mostly arms and legs.	
Progressive in its course.	
Treatment powerless.	

Tetany. (Curschmann, 1915.)

"Intentional" in some cases.	Tonic spasms.
Easily induced by stimuli.	Hands and arms mostly.
Legs often attacked.	Spasm duration long.
Idiopathic tetany incurable.	Infection indicated.

Progressive lenticular degeneration. (Wilson, 1912; Spiller,⁴ 1916.)

Bilateral.	Not congenital.
Affects both extremities.	Accompanied by cirrhosis of liver?
Increase with volitional movement.	Tonic spasticity of face and limbs.
Reflexes preserved.	
Always fatal.	
Familial.	

Aplasia axialis extra-corticalis congenita. (Merzbacher, 1908; Batten and Wilkinson, 1914.)

Congenital or in first three months.	Affects chiefly males.
Hereditary.	Slowly if at all progressive.
	Symptoms constant.
	Not so fatal.

Paramyotonia congenita. (Eulenberg, 1886.)

Congenital.	Tonic spasms.
Hereditary.	Not always bilateral.
	Last for hours.
	Apparently dominant.

It is clear that none of the above-mentioned conditions can be considered as identical with congenital palsy. The most common similarity is that several of them are known to be recessive in inheritance, but they all differ in other symptoms. Congenital palsy differs from any of the other conditions in being definitely congenital and running a brief course terminating in death at an early age.

In conclusion it may be pointed out that while the data may never be sufficiently complete in man, it may be pos-

⁴ According to Spiller the conditions attributed to disease of the lenticular nucleus are numerous, including the pseudo-sclerosis of Westphal and Strümpell, Huntington's chorea, Parkinson's disease, and a number of others.

sible in animals where breeding experiments can be conducted, to use the inheritance method for separating nervous diseases in which the symptoms are so similar as to be confusing, or even identical. For example, let us suppose that a recessive neurosis similar to congenital palsy should appear in another line of guinea-pigs. If animals heterozygous for it were mated to heterozygous individuals of our stock and they produced affected offspring in a one-to-three ratio, it would be good evidence that we were dealing with the same heritable trait in both strains. If, however, the disease in the new line was genetically different, a different ratio of offspring would be expected, presumably nine normal to seven neurotic individuals, assuming that there was no linkage of the two genes concerned. It is possible that even in man when the family histories are sufficiently complete the method of genetic analysis may help in the differentiation of neuroses characterized by symptoms which are confusingly similar.

SUMMARY

1. A definite neurosis appeared in our guinea-pig stock in 1914, characterized by clonic spasms, particularly of the legs. When in a spasm the animals lie on their sides in a helpless condition. This state is induced by various stimuli, but especially by those of a sharp auditory nature, and also by attempted volitional movements of the legs.

2. The affected animals are fully up to average weight when born, and appear to be normal in all other respects. While different individuals vary with respect to the intensity of the symptoms at birth, they are always easily distinguished from normal young, and in all cases the disease runs a short progressive course, terminating in death within about two weeks at most.

3. This defect, which we have called congenital palsy, is definitely heritable. It is a simple Mendelian recessive, and normal and affected offspring are produced by two

heterozygous parents in the ratio of three normal to one affected.

4. It has been shown that heterozygous animals mated to normals produce offspring of the same classes as themselves in equal numbers. Furthermore, it has been proven that homozygous dominants can be extracted from heterozygous parents and that they show no more tendency to transmit the disease than individuals of normal unrelated stock.

5. Heterozygous animals are entirely normal in their reaction and can be told from the homozygous only by breeding tests.

6. A survey of the literature relating to nervous defects in man and other animals does not reveal any condition corresponding exactly to congenital palsy. Some of the conditions in pigeons, rodents and in man are similar in that they are recessive in inheritance.

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ANIMAL LIFE AND SEWAGE IN THE GENESEE RIVER, NEW YORK*

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It is a hopeful sign of permanent improvement in our rivers and streams when commonwealths and municipalities turn their attention to the condition of these waters and provide means for their purification where they have previously been contaminated by sewage, refuse, or chemicals.

It has been known to biologists for many years that sewage and chemicals were inimical to the life inhabiting these waters, but political bodies have been slow to realize or to admit that the pouring of millions of gallons of crude sewage had any effect on the animal life living in such waters. It is even probable in some cases that those in authority cared little about the effect of such contamination, if it provided an easy and economical method of disposing of the sewage. The damage to fish and other aquatic life has not been realized by the engineers in charge of such work and hence this class of scientific men has not protested or sought a better method, at least not until recent years. The work of the various conservation commissions of the several states, as well as the efforts of natural history societies, universities, and private individuals, have brought into prominence the danger from stream pollution and have awakened widespread interest in this important subject.¹

In Illinois, careful studies are in progress by the Nat-

* Contribution from the Museum of Natural History, University of Illinois.

¹ See in this connection, Henry B. Ward, "The Elimination of Stream Pollution in New York State," *Trans. Amer. Fisheries Soc.*, XLVIII, pp. 1-25, 1918.

ural History Survey under the direction of Dr. S. A. Forbes, for the purpose of ascertaining the effect in the Illinois River of the large volume of polluted water from the Chicago Drainage Canal, into which all of the sewage of the city of Chicago is discharged.² In other places, studies of a similar character are being carried on.

In New York State, the Genesee River, at Rochester, has afforded a striking example of stream pollution, of the effect of this pollution on the animal life in the river, and of the final return of this life after the amount of pollution was notably reduced. It has been the writer's good fortune to visit Rochester every two or three years (sometimes oftener) and to be able to study the condition of the Genesee River during a period of nearly thirty years. Collections were made before, during, and after pollution, permitting comparisons to be made of the life in the river during these several periods of varying conditions.

The animal life in a body of water has been little used as an indicator of the degree of pollution. Fish, especially young fish, have been used and are good indicators because they cannot live in water polluted to any large degree. The relative resistance of different species of fish has been well shown by Shelford in a recent paper.³ The writer is convinced that mollusks are also good indicators of degrees of pollution. The intimate relation of fish to the propagation of river mussels (*Unionidæ*), so largely used in the manufacture of pearl buttons, is also seriously affected by stream pollution.

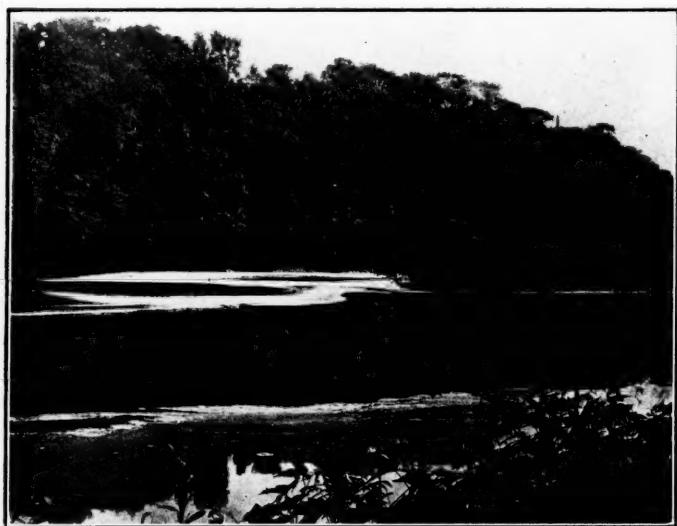
The polluted portion of the Genesee River studied (which was also the place of maximum pollution) lies below the lower falls (Driving Park Avenue bridge), a large sewer discharging some distance below these falls just above the spot known locally as Brewer's landing, near Norton Street, on the east side of the river. Several

² See Forbes and Richardson, "Some Recent Changes in Illinois Biology," Illinois Natural History Survey, *Bulletin*, XIII, pp. 139-156, 1919.

³ *Bull.* XIII, Ill. Nat. Hist. Surv., pp. 25-42, 1918.

other sewers emptied into the river at the north end of Maplewood Park on the west side of the river. The stream for several miles below these points resembled thick, dirty, greasy dish water, a heavy scum covering the surface of the water as well as the shore and any objects in the water.

At the time of maximum discharge, sewer outlets also entered the river both above and below the falls and



The photograph was taken in the summer of 1907. The white streaks in the background are from the trunk line sewer on the east side of the river near Norton Street. The light streak in the foreground is the sewage from the outlet on the west side of the river near the lower falls. A small island on the right side of the picture hides a part of the sewage in the water on the east side of the river. This place is six miles up the river from the mouth.

many manufactories also contaminated the water by discharging chemicals and refuse into the river. At the present time there are no sewers entering the river above the falls. There are two sewer outlets at the northern end of Maplewood Park, one from the residence section west of the river (Dewey Avenue section) and one from the Eastman plant. Sewage is also discharged into the

river at Charlotte near the mouth of the river. This, however, does not enter into the present discussion. It has not been possible to ascertain the amount of sewage entering the river during the period of maximum discharge, but at the present time the approximate quantity of sewage discharged is one-eighth cubic foot per second (data from Rochester engineering department). This is a comparatively small amount which apparently has little or no effect on the animal life in the river. On the contrary, it may provide food for some organisms.

Sewage was turned into the Genesee River about the year 1820. Collections of molluscan life were made in July, 1897, and nine species were obtained, as noted below:

<i>Musculium transversum</i>	<i>Physa gyrina</i>
<i>Musculium partumeium</i>	<i>Physa sayii</i>
<i>Bythinia tentaculata</i>	<i>Physa heterostrophia</i>
<i>Galba catascopium</i>	<i>Galba caperata</i> (rare)
<i>Planorbis trivolvis</i>	

Previously, in 1892-1895, collections had been made which included about the same species as noted above. Individuals were notably abundant, thickly covering the rocks and the shore. At the time the above mollusks were collected it was noted that the sewage was increasing in volume and pollution was becoming more noticeable. It was predicted at this time that in a few years the fauna would be exterminated by the foulness of the water.

The river was visited and examined in 1898, 1900, 1901, 1904-1907, 1908, 1910, 1913, 1915-1917, and 1919.⁴ Each year it was noted that the pollution of the water was rapidly increasing. In 1907, the water-breathing mollusks, *Musculium* and *Bythinia*, had succumbed and none could be found. The air-breathers, *Galba*, *Planorbis*, *Physa*, still held out, though reduced in number of indi-

⁴See the following papers by the writer: "The Molluscan Fauna of Western New York," *Trans. Acad. Sci. St. Louis*, VIII, pp. 71-94, 1898. "The Molluscan Fauna of the Genesee River," *AMER. NAT.*, XXXV, pp. 659-664, 1901.

viduals. An examination made in 1910 failed to discover a single living mollusk of any species. Apparently, the water had reached such a state of concentrated pollution that even the air-breathing mollusks, which normally come to the surface to take in free air, could not adapt themselves to this unfavorable environment and were either killed or compelled to migrate down the river to a point where the pollution was not so great, a distance of several miles. During the following two or three years the river was visited but no mollusks could be found.

On March 17, 1917, a large part of the city's sewage was diverted to the Irondequoit sewage disposal plant located on the shore of Lake Ontario near the Durand-Eastman Park. Here an average of 32 million gallons of sewage are treated daily, and the treated sewage discharged into Lake Ontario at a distance of 7,000 feet from shore in water 50 deep (vide city engineer's statement). It may easily be seen that when this large amount of sewage, untreated, was discharged into the Genesee River, it could not but render the water totally unfit for animal life and a menace even to the inhabitants who visited the beautiful parks bordering both sides of the river.

The result of the diminution of the amount of sewage discharged into the river has been that the fauna has returned and has rapidly taken possession of the favorable environments which were in use previous to the maximum period of pollution. Collections made in September, 1919, contained the six species noted below:

Musculium transversum *Bythinia tentaculata*

Planorbis trivolvis *Galba catascopium*

Physa integra

Physa oneida (previously reported as *heterostrophia*)

It will be noted that practically the same species returned to the Maplewood Park section of the river that lived here before the polluted water exterminated the fauna, indicating, probably, that they migrated up the river from the less affected water below.

Few data are at hand indicating how far the polluted water must flow before it can purify itself enough to become favorable for animal life. In the Illinois River, life is being affected by the Chicago sewage at Peoria, a distance of 110 miles from the source of infection. A recent study of the Salt Fork of the Vermilion River, into which the sewage of Champaign and Urbana is discharged, indicated that the polluted water was inimical to molluscan life for a distance of 14 miles in which no living mollusks were found, and one must pass down the stream for a distance of nearly twenty miles before a normal mussel fauna can be found.

In the Allegheny River, Ortmann found that whole stretches of the stream and some of its tributaries had been made into a desert by pollution, principally in the form of chemicals from the numerous mines situated in this part of the State. Ortmann remarks that "with regard to the animal life in our rivers, sewage does not seem to be harmful; on the contrary, certain forms (fishes, crawfishes, mussels) seem to thrive on it" (p. 97). This is probably true in a case where water is but slightly contaminated; but in streams where pollution by sewage is greatly concentrated (a condition reached sooner or later in all streams used for sewage disposal) it is certainly inimical to the forms of life mentioned. It is very true that a stream polluted by chemicals soon becomes destitute of the larger forms of animal life (if, indeed, not all life) and in such waters the return of life will be very slow and in many cases it may be impossible for life to return on account of the chemicals which cover the bottom and shores.

In the case of the Genesee River, we have a striking example of the history of a polluted stream and its effect on the animal life. Previous to the discharge of sewage into the stream there was a varied molluscan fauna very numerous in individuals. In the course of eleven years the gill-bearing mollusks were forced out and after a lapse of fourteen years all molluscan life ceased to live

in this portion of the river. Seven years later, the greater amount of sewage was diverted to another outlet. Two years after this diminution of pollution we find that the molluscan fauna has returned in as great number of individuals as were found there before pollution began. In other words, it required but two years (possibly less, as the river was not examined in 1918, one year after the conditions changed) for the river to become pure enough to provide a favorable environment for molluscan life. It has been reported that the sturgeon is again resorting to the lower portion of the river for spawning purposes, after an absence of several years, due to the heavy pollution of the water. The rapid return to a favorable condition is partly due to the lower falls in the river which abundantly aerates the water before it is mixed with the small amount of sewage now flowing into the stream.

It may be affirmed without successful contradiction that wherever sewage pollution occurs, sooner or later the animal life will be affected, and finally driven out. As this condition seriously concerns our food and game fishes, which form so large a part of the meat food of our population, it is a situation that demands immediate attention and early remedy. That the fauna recovers so quickly after pollution ceases is a matter of great interest and satisfaction, showing what favorable returns may be expected when these matters are taken up in all earnestness by municipalities and commonwealths.

POLLUTION IN THE GENESEE RIVER

Since writing the above account of the effect of sewage pollution on the molluscan life of the river, Mr. John F. Skinner, principal assistant engineer of the Rochester Department of Engineering, who has been connected with this department for upwards of twenty-eight years, has kindly read the paper and has indicated several inaccuracies in the historical matter, besides adding much information of value concerning the sewage disposal of the city. The following data are all supplied by Mr. Skinner:

Rochester was settled in 1812, incorporated as a village in 1817, and as a city in 1834. The first sewers were built about 1820. All of the city west of the river and roughly everything within a mile east drained into the river. A sewer 4 by 6 feet in diameter was in operation in 1863. In 1896 nine main outfalls were in operation, five on the west side and four on the east side. In 1897 the west side trunk sewer nine feet in diameter was put in operation. Four of these sewer outfalls are below the lower falls, two being above the point at which the molluscan studies were made.

Fourteen stormwater sewers overflow into the river above the lower falls. Refuse and waste matter, both liquid and solid, enter the stream from a tannery, gas works, breweries, garbage disposal plants and some other manufacturing plants. The breweries do not now contaminate the water as formerly. This additional pollution is sometimes more harmful to animal life than the sewage itself. In March, 1917, the main (Irondequoit) sewage disposal plant was put in operation on the shore of Lake Ontario. The outfall to this plant intercepts the dry weather flow of all of the sewer outlets mentioned above, except the Lake Avenue or Dewey Avenue outlet on the west side about 6,000 feet below the lower falls. There is also a large outlet further north, down the river, from the Eastman Kodak plant and adjacent territory. The overflows from the east and west side trunk sewers enter below the lower falls, as will also that from the Lake Avenue sewer after the Maplewood plant is completed. The clarified effluent of the last mentioned plant will also enter the river, but the major portion of the solids (contained in the sludge) will be pumped across the river to the east side interceptor.

In a report issued in 1913,⁵ Mr. George C. Whipple, consulting engineer, has published much valuable information relating to the effect of the sewage pollution on

⁵ "Report on the Sewage Disposal System of Rochester, N. Y.," by Edwin A. Fisher, city engineer. See pages 179-200.

the river and on some of the animal and vegetal life. This study was made in 1912 when the pollution was at its maximum and during the period when molluscan life had disappeared from the upper part of the river below the lower falls. The dissolved oxygen in the lower river, below the trunk line sewer, in July and August when the temperature was high and the water low, varied from 5 to 41 per cent. of saturation. The water at the bottom of the river almost always contained less oxygen than that at the surface. This condition prevailed to within a short distance of the mouth of the river when the reverse was true, this change being due to the backflow of the well-oxygenated water from Lake Ontario. Near the east side trunk sewer, which is about half a mile below the lower falls, the percentage of saturation varied from 5 to 60 between July 1 and August 13. On August 13, the percentage of saturation between the east side trunk sewer and a point two and a half miles from the lake (a distance of about three miles) did not exceed five per cent. This area includes the shores examined for the Mollusca. The percentage of dissolved oxygen saturation was usually higher at the surface than at the bottom of the river, the heavier parts of the sewage falling to the bottom and forming sludge banks. The percentage of dissolved oxygen also did not vary directly with the amount of evident pollution, for on a day in July when the most disagreeable conditions existed for a mile and a half below the east side outlet the dissolved oxygen at the surface varied from 40 to 70 per cent.

A study of the plankton of the river indicated that near the source of pollution, $5\frac{1}{2}$ miles above the mouth of the river, there were on the average in July and August, 1,650,000 bacteria, 156 algæ, 209 Protozoa, and 57 Crustacea and Rotifers per cubic centimeter. At the mouth of the river the figures for these organisms per c.c. stood as follows: 67,000; 363; 77; 233. It is unfortunate that no discrimination was made between the foul water algæ and protozoa and those normally inhabiting pure water,

which would have made a difference in the number from the standpoint of pollution. This has been done by Forbes and Richardson in their studies of the Illinois River pollution.⁶

A comparison of the report made by Engineer E. Kuichling, Feb. 1, 1907 (1913 report, pp. 5-42) with that of Mr. Whipple made in 1912 shows in a striking manner the rapid increase of polluted conditions, the former author describing conditions as not very bad (pp. 10-11) while the latter author, five years later, describes the conditions as very bad (p. 182). It was between these dates, 1907 and 1912, that the molluscan fauna disappeared and it is apparent that the distinct increase in toxicity is indicated from these several angles of vision.

It should be stated in connection with the ill effects of sewage pollution that it affects the population in an indirect manner not usually recognized by sanitary engineers who have not interested themselves in the problem of fish culture. Such places as the six miles of shallow shore bordering the Genesee River are the breeding and feeding ground of such valuable food and game fish as the sturgeon, black bass, sunfish, suckers, bullheads, pickerel, pike, etc., and the young of these and other fish spend a large part of their life in this kind of a habitat, to later migrate into the open lake.

⁶"Studies on the Biology of the Upper Illinois River," *Bull. Illinois State Laboratory of Natural History*, IX, pp. 481-574, 1913.

ALTERNATIVE EXPLANATIONS FOR EXCEPTIONAL COLOR CLASSES IN DOVES AND CANARIES

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DOVES and canaries have been shown to possess certain color factors which are sex-linked in inheritance. The behavior of these factors leads one to the conclusion that, unlike *Drosophila*, cats and man, the female and not the male is the homozygous sex. In this respect they resemble *Abraxas*, poultry and the domestic pigeon.

In both doves and canaries, however, there occur exceptional color classes in certain matings where the sex-linkage of the factors in question manifests itself. To explain the appearance of these exceptional color classes, Sturtevant (1912) (in canaries) and Bridges (1913) (in doves) have suggested that the principle of *partial sex-linkage* is involved.

Later, Bridges (1916), in discussing the phenomena of *non-disjunction* in *Drosophila*, has reviewed briefly other forms to which he considers non-disjunction may apply and among these mentions doves and canaries as follows:

(Doves—p. 157) Exceptions to the inheritance of blond and the dark types of pigeons have been explained as partial sex-linkage (Bridges, 1913) but non-disjunction offers an alternative hypothesis which seems plausible.

(Canaries—p. 158) Exceptions to sex-linkage in the inheritance of pink versus black eye colors have been reported (Durham and Marryatt, 1908). These exceptions are explainable by non-disjunction or by partial sex-linkage.

In this paper an attempt will be made to show that the hypothesis of partial sex-linkage and of non-disjunction expect certain results from the crosses made, which have not been reported and in the case of non-disjunction might, in addition, be fairly considered as involving sterility of certain color classes—a phenomenon not yet reported in any of the forms in question.

A further effort will be made to explain the observed facts on an hypothesis of *factorial change* which involves neither a break

in sex-linkage nor non-disjunction, and which expects no unusual sterility nor the appearance within the crosses made, of other unrecorded exceptions to the normal relation between phenotypic color classes.

We may take up in order the three hypotheses of partial sex linkage, non-disjunction and factorial change and may compare them on the basis of the experimental results obtained.

PARTIAL SEX-LINKAGE

1. *Doves*.—The normal result obtained when white male and colored female ring doves are crossed, is colored males and white females. This may be explained as follows: Let *W* equal a factor for the production of colored plumage and *w*, a factor allelomorphie to it for the production of white plumage. The male is *FFMM*, the female *FFMm* in formula. *W* is linked with *M* in inheritance.

White Male <i>wwFFMM</i>	Colored Female <i>WwFFMm</i>
Forming gametes <i>wFM</i>	<i>WFM</i> and <i>wFm</i>
Zygotes obtained: <i>WwFFMM</i> = colored males	
<i>wwFFMm</i> = white females	

In addition, however, exceptional colored females are produced infrequently and have been recorded by Staples-Browne (1912), and by Strong (1912). These exceptional colored females have been accounted for by Bridges (1913), as follows:

If in the female the sex-differentiating factor and the factor for plumage color are placed close enough together in the same chromosome to be linked, but not so close that linkage is complete "crossing-over" would cause the two factors which entered in the same member of the homologous pair of chromosomes to lie in different members and hence to segregate to different gametes.

If this occurred we should have the following condition:

White Male <i>wwFFMM</i>	×	Colored Female <i>WwFFMm</i>
Forming gametes <i>wFM</i>		<i>WFM</i> <i>wFm</i> } commonly <i>wFM</i> <i>WFm</i> } exceptionally
Zygotes formed: <i>WwFFMM</i> colored male		} commonly
<i>wwFFMm</i> white female		
<i>WwFFMm</i> colored female		
<i>wwFFMM</i> white male		} exceptionally

This would account for the exceptional colored females, reported from this cross by Staples-Browne and by Strong. It also expects an additional exceptional class—namely white males, and these, though they should occur as frequently as do the colored females, are conspicuous by their absence.

Further than this the hypothesis as just outlined supposes that "crossing over" occurs in the heterozygous sex, between chromosomes which correspond to the X and Y chromosomes of *Drosophila*. This condition has not been observed in *Drosophila* or in forms where a similar opportunity exists and it must therefore, be considered as entirely hypothetical and contrary to such evidence as the most extensively investigated forms have given.¹

2. *Canaries*.—In this form, the sex-linked inheritance of the factor for dark-eyed color (P) having as an allelomorph pink eye color (p) has been demonstrated by Durham and Marryatt (1908) and reviewed by Sturtevant (1912). Here, however, as in doves there is an unexpected color class which makes its appearance. The exceptional individuals are dark-eyed females which occur in a cross between pink-eyed males and dark-eyed females where only dark-eyed males and pink-eyed females are expected.

Sturtevant, in reviewing the case and in attempting to explain it as the result of partial sex linkage says (p. 570):

This hypothesis could be easily tested. If it is correct, then the cross just discussed should, if large enough numbers be reared, produce as many pink-eyed males as black females." The occurrence of such pink-eyed males has not been reported although it seems almost certain that their appearance in this cross would have been observed and mentioned by breeders did they occur even very rarely.

We may, therefore, say that should the missing color classes appear in the dove and canary matings as predicted by the hypothesis of partial sex linkage, that hypothesis would have a

¹ Cole and Kelley (1919) have studied the linkage relations between two sex-linked factors in the domestic pigeon and have on the basis of considerable data, come to the conclusion that "crossing over" occurs in the male but not in the female. This result seriously invalidates partial sex-linkage as a possible explanation for the exceptionally colored females in doves or dark-eyed females in canaries. Furthermore, Goodale (1917) has shown that "crossing over" has occurred in the male and not in the female of the domestic fowl—a point which also has a direct bearing on the work with doves and canaries.

stronger claim to recognition as the correct one for explaining the observed phenomena. Until such time, however, the explanations of the observed exceptions by an hypothesis which requires the appearance of an approximately equal number of exceptions in the same cross, without any evidence that such exceptions exist can not be considered as satisfactory. That this is the case for doves has been suggested by Bridges in his more recent (1916) paper on non-disjunction as already quoted.

NON-DISJUNCTION

In 1916 Bridges, in giving the data on which rests most of the experimental proof of the existence of non-disjunction in *Drosophila*, suggested that the exceptions to sex-linked inheritance in doves and canaries, might be the result of non-disjunction of the sex chromosome.

If, however, the matings producing such exceptional individuals are analyzed on the basis of non-disjunction, certain discrepancies between expectations and actual results become evident. These discrepancies suggest some fundamental difficulties in applying the hypothesis of non-disjunction to the cases in question. Thus if we assume as does Bridges that the sex formula in both doves and canaries is FFMM in the male and FFMm in the female we may represent the crosses made as follows: Theoretically there may be either (a) no non-disjunction, or (b) non-disjunction in the male, or (c) non-disjunction in the female. In each mating we shall consider the three possibilities:

1. In doves:

White Male \times Colored Female

	White Male wWFFMM	\times	Colored Female WwFFMm
(a) If no non-disjunction; forms gametes	wFM		$\left\{ \begin{array}{l} WFM \\ wFm \end{array} \right.$
(b) If non-disjunction in the male; forms gametes	$\left. \begin{array}{l} wwFFMM \\ \text{and } - \end{array} \right\}$		$\left\{ \begin{array}{l} WFM \\ wFm \end{array} \right.$
(c) If non-disjunction in the female; forms gametes:	wFM		$\left\{ \begin{array}{l} WwFFMm \\ \text{and } - \end{array} \right.$

In the three cases the zygotes formed will be as follows:

(a) If no non-disjunction:

- (1) WwFFMM = colored males
- (2) wwFFMm = white females

This is the usual result obtained with, however, the addition of exceptional dark females which we are trying to explain.

(b) If non-disjunction in the male: forms the following zygotic classes:

- (1) WwWFFFMMM = males? Colored? Probably die.
- (2) WFM — = colored females? Sterile?
- (3) wwWFFFMMm = white males (transmitting non-disjunction).
- (4) wFm — = whites? Probably die.

It should be noted that non-disjunction in the male of *Drosophila* does not occur. Yet in pigeons the male is presumably the "homozygous" sex and this makes its chromosome condition in respect to sex more closely analogous to the female *Drosophila* in which primary non-disjunction does occur. The classes b(1) and b(4) we may fairly suppose, fail to survive. The triple X condition of form b(1) is fatal in *Drosophila* as is the absence of both X and Y seen in class b(4). The two classes b(2) and b(3) are however, real difficulties. The sterility of class b(2), the exceptional colored females has never been reported, as would undoubtedly have been the case, did it exist—just as their appearance alone has excited comment and interest. Class b(3), moreover, would certainly be white males and as we have already seen there is no record of any such animals appearing in this cross. Since these white males are the means of transmitting the tendency for non-disjunction to further generations, their presence is necessary for the occurrence of secondary non-disjunction and their absence is a serious handicap to the acceptance of the hypothesis in this material.

(c) Non-disjunction in the female:

- (1) WwWFFFMMm = colored males (transmitting non-disjunction).
- (2) wFM — = white females? Sterile?

It will be seen that the exceptional colored females are neither expected nor explained by this type of non-disjunction. Unless, therefore, we are to assume that non-disjunction in doves is, in almost all its fundamentals, different from the same process in *Drosophila*, producing qualitatively different results, we must agree that it fails to meet the experimental facts. If we do suppose that it differs fundamentally, it may fairly be claimed that no evidence of a conclusive nature either cytological or genetic exists to lead us to say that non-disjunction is in any way involved.

Although the cross just considered is the one chiefly cited, it is of interest to attempt to apply the three possibilities in question to the reciprocal cross namely colored male \times white female—as follows:

	Colored Male WWFFMM	\times	White Female wwFFMm
(a) If no non-disjunction; forms gametes:	WFM		$\left\{ \begin{array}{l} wFM \text{ and} \\ wFm \end{array} \right.$
(b) If non-disjunction in the male, forms gametes:	WWFFMM and	$\left\{ \begin{array}{l} wFM \text{ and} \\ wFm \end{array} \right.$	$\left\{ \begin{array}{l} wFM \text{ and} \\ wFm \end{array} \right.$
(c) If non-disjunction in the female, forms gametes:	WFM		$\left\{ \begin{array}{l} wwFFMm \text{ and} \\ - \end{array} \right.$

(a) No non-disjunction. A normal mating of this type gives two classes of offspring as follows:

- (1) WwFFMM = colored males.
- (2) WwFFMm = colored females.

No exceptions have been recorded.

(b) Non-disjunction in the male: would expect four types of zygotes as follows:

- (1) WWwFFMM = colored males? Probably die.
- (2) WWwFFMm = colored males—transmitting non-disjunction.
- (3) wFM — = white females? Sterile?
- (4) wFM — = whites? Probably die.

Did class b(3), sterile white females occur, their appearance would undoubtedly have been noted and recorded. It should further be noted that non-disjunction, namely in the male, is the only type able to account for the appearance of exceptional colored females in the cross reciprocal to that just considered. In this cross, however, this type of non-disjunction expects a color class which has not been recorded in matings of supposedly homozygous colored males.

(c) Non-disjunction in the female:

- (1) WwwFFMMm = colored males transmitting non-disjunction.
- (2) WFM — = colored females? Sterile?

Here, non-disjunction gives no exceptions save the occurrence of an occasional sterile colored female. This would undoubtedly

be able to escape detection by breeders unless the most careful individual records were kept. However, non-disjunction in the female fails entirely to account for the occurrence of the observed exceptions in the reciprocal cross and may for that reason be disregarded.

Instead, therefore, of increasing the probability that non-disjunction is involved in the production of the exceptions noted, a consideration of the above cross shows that it either fails to account for the exceptions which *do* occur or else expects additional color classes which have not been observed.

2. *Canaries:*

Here the conditions differ but slightly from those already described for doves. The factors involved are P, dark eye color epistatic to p, pink eye color. P is commonly considered as sex linked. The possibilities of non-disjunction remain the same as in doves and may, therefore, be taken up under parallel headings.

Cross of Pink-eyed Male \times Dark-eyed Female.

	Pink-eyed Male ppFFMM	\times	Dark-eyed female PpFFMm
(a) If no non-disjunction; forming gametes	pFM		PFM and pFm
(b) If non-disjunction in the male: forming gametes	ppFFMM and —		PFM and pFm
(c) If non-disjunction in the female: forming gametes	pFM		PpFFMm and —

The zygotes formed by these three processes would, in order, be as follows:

(a) No non-disjunction:

- (1) PpFFMM = dark-eyed males.
- (2) ppFFMm = pink-eyed females.

It is in this cross that exceptional dark-eyed females sometimes occur.

(b) Non-disjunction in the male:

- (1) PppFFFMMM = probably dies.
- (2) PFM — = dark-eyed females? Sterile?
- (3) pppFFFMMm = pink-eyed males transmitting non-disjunction.
- (4) pFm — = probably dies.

Here, as Durham and Marryatt who reviewed the case have stated, although canary breeders have long noticed the occur-

rence of dark-eyed females, *no mention is made of their sterility nor of the occurrence of Class b(3), pink-eyed males, although the latter should have appeared with approximately equal frequency.* When one considers the amount of canary breeding which has been done, and is still being continued and the fact that breeders have long recognized the exceptional dark females, the continued absence of the expected pink-eyed males becomes a real objection to the acceptance of any hypothesis which calls for their appearance.

(c) Non-disjunction in the female:

- (1) PppFFFMm = dark-eyed males transmitting non-disjunction.
 (2) pFm — = pink-eyed females? Sterile?

It will be noted that this type of non-disjunction fails, as it did in doves, to account for the observed exceptional dark-eyed females.

Further if we now consider the reciprocal cross of dark-eyed male \times pink-eyed female, we shall find that the only type (b) of non-disjunction which is able to account for the exceptional color class above recorded demands a type of result as yet not reported.

	Dark-eyed Male PPFFMM	\times	Pink-eyed Female
(a) If no non-disjunction; forms gametes	PFM		{ ppFFMm pFM and pFm
(b) If non-disjunction in the male; forms gametes	PPFFMM and } — }		{ pFM and pFM
(c) If non-disjunction in the female; forms gametes	PFM		{ ppFFMm and —

The following classes of zygotes will be expected:

(a) If no non-disjunction:

- (1) PpFFMM = dark-eyed males.
 (2) PpFFMm = dark-eyed females.

No exceptions have been recorded.

(b) If non-disjunction in the male:

- (1) PPpFFFFMM = dark-eyed? Probably dies.
 (2) PPpFFFFMm = dark-eyed males transmitting non-disjunction.
 (3) pFM — = pink-eyed females? Sterile?
 (4) pFm — = probably dies.

Here it will be observed that pink-eyed females, probably sterile, should be produced as frequently as were the exceptional dark females in the reciprocal cross. We have no evidence that this is the case.

(c) If non-disjunction in the female:

- (1) PppFFFMMm = dark-eyed males transmitting non-disjunction.
- (2) PFM — = dark-eyed females? Sterile?

Here, as in doves, the result would possibly be masked because no unusual color type is expected. Since, however, this form of non-disjunction would fail to account for the dark females in the cross of pink-eyed male \times dark female it may be disregarded.

To sum up, we may say that non-disjunction is able to explain part of the observed facts but expects sterility and other exceptional color classes in crosses where they have not been found. If then, an explanation can be found which expects, in the crosses made, the observed color classes and none other, it should in the absence of stronger evidence for non-disjunction, be considered to be fully as likely an explanation of the phenomena observed.²

FACTORIAL CHANGE

1. *In Doves*.—In a paper now in press, I have attempted to show that the occurrence of exceptional color classes in cats (other than tortoise-shell males) which have been variously interpreted as due to partial sex-linkage or to the action of modifying factors, may be satisfactorily explained by a process of factorial change. Thus if in cats in some of the gametes of certain unusual individuals the factor Y for the restriction of black pigment from the coat, appeared in its hypostatic and allelomorphic form y, the exceptional color classes would be accounted for. If a somewhat similar process occurred in certain rare individuals in doves, between factors W and w, but in the reverse direction, namely, from w white to W colored, we should have an explanation for the exceptions observed.

If, then certain white male doves formula wwFFMM formed

² Cole and Kelley (*loc. cit.*) believe that the exceptional colored females in crosses between white male and colored female doves are simply mistakes in observation or records. Because of the fact that they were obtained by two entirely independent investigators and because a similar exception is found in the case of canaries where even more extensive evidence exists, it is believed that the case demands explanation and cannot be merely disregarded as Cole and Kelley imply.

among their gametes some that were WFM instead of wFM the following result would be obtained in a cross between one of them and a colored female.

	White Male wwFFMM	×	Colored Female WwFFMm
Forming gametes:	wFM commonly WFM exceptionally		WFM wFm
Zygotes expected:	(1) WwFFMM = colored males (commonly) (2) wwFFMm = white females (commonly) (3) WWFFMM = colored males (exceptionally) (4) WwFFMm = colored females (exceptionally)		

Of the two exceptional zygotic classes (3) and (4) only the latter represents a distinguishable phenotypic difference. The other (3) would be merely individuals homozygous for the factor W and therefore indistinguishable, except by proper breeding tests, from the common heterozygous class (1). Such tests have not been reported on as yet by investigators in whose stock, class (4) individuals have appeared. The point to be emphasized, however, is that the presence of homozygous colored males might very easily escape notice unless sufficient numbers of young from a critical cross were raised. It should further be noted that no sterility is expected nor has any been recorded.

If a similar change occurred at rare intervals in certain *white females* as we have a right to expect it possibly would, we should have *no phenotypically aberrant or unexpected color classes formed* in crosses between such females and colored males. We should however, obtain infrequently as a result of this process *homozygous* instead of heterozygous colored males and peculiarly constituted colored females as follows:

	Colored Male WWFFMM	×	White Female wwFFMm
Forming gametes:	WFM		{ wFM commonly wFm WFM exceptionally WFm
Zygotes expected:	(1) WwFFMM = colored males } (2) WwFFMm = colored females } commonly. (3) WWFFMM = colored males } (4) WWFFMm = colored females } exceptionally.		

Here again only careful individual breeding tests would be expected to reveal the presence of the exceptional homozygous

colored individuals of classes (3) and (4) and no sterility above the ordinary would be expected.

It is also interesting to note that theoretically the colored females of class (4) would when crossed with ordinary white males yield *colored females* of the normal type. Thus:

	White Male	×	Colored Female Class (4)
	wwFFMM		WWFFMm
Forming gametes:	wFM		WFM
			WFm
Zygotes expected:	(1) WwFFMM = colored males.		
	(2) WwFFMm = colored females.		

It may be objected that changes from a hypostatic factor to its epistatic allelomorph are not frequent. This is admitted. On the other hand, they have been several times reported by investigators, among others by Morgan, in *Drosophila*, and by the writer, in mice. In this connection it is interesting to note that the white doves referred to are not totally unpigmented being merely dilute, a fact easily observed in their eye color and found by Strong (1912) to hold true for their plumage.

2. In *Canaries*.—The factors involved are the allelomorphic P for dark eye color and p for pink-eye color. The quantitative relation between the two is somewhat similar to that described in doves though considerably less marked. The factorial change appears to be extremely rare and to be from the p to the P condition. The following results would be expected if the change occurred in the pink-eyed male.

	Pink-eyed Male	×	Dark-eyed Female
	ppFFMM		PpFFMm
Forming gametes:	pFM commonly		{ PFM PFm
	PFM exceptionally		
Zygotes expected:	(1) PpFFMM = dark-eyed males	}	commonly
	(2) ppFFMm = pink-eyed females		
	(3) PpFFMM = dark-eyed males	}	exceptionally
	(4) PpFFMm = dark-eyed females		

Here as in doves the homozygous and heterozygous dark-eyed males would be distinguishable only after a carefully controlled breeding test. The dark-eyed females would be the only exceptional phenotypically distinct color class expected.

If the change occurred in the dark-eyed female instead of in the pink-eyed male we should have the following condition.

	Pink-eyed Male ppFFMM	×	Dark-eyed Female PpFFMm	
Forming gametes	pFM			
			PFM	} commonly
			pFm	
			PFM	} exceptionally
			pFm	
Zygotes expected:	(1) PpFFMM = dark-eyed males			} commonly
	(2) ppFFMm = pink-eyed females			
	(3) PpFFMM = dark-eyed males			} exceptionally
	(4) pPFFMm = dark-eyed females			

Here again *dark-eyed females* are the only unusual phenotypic color class produced.

In the reciprocal cross we should have the following:

	Dark-eyed Male PPFFMM	×	Pink-eyed Female ppFFMm	
Forming gametes:	PFM			
			pFM	} commonly
			pFm	
			PFM	} exceptionally
			pFm	
Zygotes expected:	(1) PpFFMM = colored males			} commonly
	(2) PpFFMm = colored females			
	(3) PPFFMM = colored males			} exceptionally
	(4) PPFFMm = colored females			

As in doves the dark-eyed females of class (4) would be expected when crossed with ordinary white males, to produce *dark-eyed females*, an unusual color class, as well as dark-eyed males, a usual one.

It will then be seen that the hypothesis of factorial change accounts for all the observed facts and unlike the hypothesis of partial sex-linkage or that of non-disjunction expects neither exceptional phenotypically distinct color classes as yet not obtained, nor any exceptional degree of sterility.

On the hypothesis of factorial change it should be possible to obtain at rare intervals colored doves from white parents. None of those who reported exceptional colored females have reported this event. Nor have breeders of pink-eyed canaries recorded a dark-eyed bird from a pink-eyed × pink-eyed mating. In neither case, however, have a considerable number of young from such matings been reported by breeders *from animals of the same stock as that which gave the exceptional dark females*. It therefore remains quite probable that such a result could and would be ob-

tained as it was in Morgan's white-eyed flies and in my gray-bellied agouti mice.

Further, it would appear that another possibility, visionary though it may be, exists. If factorial change within a given locus is in any way influenced by other genes or combinations of genes within the cell either during gametogenesis or immediately after fertilization, we should expect that the *w* or *p* gene, as the case might be, would be subject to different intra-cellular environment when its allelomorph *W* or *P* was present, from that in which it would be placed in a homozygous *ww* or *pp* individual. Some of the differences which are bound to exist might well make for its relatively greater instability in the former as compared with the latter case.

*Although such a relationship is highly hypothetical, it is suggested that we should be continually on the alert for evidence of possible effects of intergenic and intra-cellular environment as one of the most probable causes of genetic change.*³

CONCLUSION

It is believed that the extension of the hypothesis of partial sex-linkage and of non-disjunction, the effects of which have been clearly demonstrated in *Drosophila* should be made to include other forms only after confirmatory genetic and, wherever possible, cytological evidence have been obtained, and in the absence of any other hypothesis which equally fits experimental facts and is capable of experimental proof.

It is therefore suggested that the occurrence of occasional colored females in a cross between white male and colored female

³ It should be recognized that sex-linked inheritance gives opportunity for the recognition of factorial changes should they occur, to a far greater extent than ordinary crosses—for example: In a case *not* involving sex linkage we cross an individual homozygous or heterozygous for *W* with a *w* individual, the small *w* might change to its epistatic allelomorph *W* in rare cases without being recognized unless each of the supposedly *Ww* zygotes resulting from the cross were tested individually and sufficient young obtained to determine whether they are exceptional *WW* individuals. This has not been done on any very large scale with either birds or mammals under experimental conditions.

If, however, the change occurred in a cross involving sex linkage, it would be at once evident in at least one type of mating. This, as we have seen, is the cross of white *wwFFMM* male by *WwFFMm* colored female where any change in the *w* factor in the male would at once become evident by the production of colored females otherwise not expected.

doves, and of dark-eyed females in a cross between pink-eyed male and dark-eyed female canaries may be due to a rare factorial change from the factor *w* to its allelomorph *W* in doves and from the factor *p* to its allelomorph *P* in canaries. Such a change would account for the observed results, except no sterility nor additional unrecorded phenotypes and would be subject to experimental tests.

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SHORTER ARTICLES AND DISCUSSION

TRICHOMONAS AND BLACKHEAD IN TURKEYS

IN reading the introduction to Dr. E. E. Tyzzer's contribution in the May issue of the *Journal of Medical Research* entitled "Developmental Stages of the Protozoon of 'Blackhead' in Turkeys," one is almost certain to be left with the impression that the conception of the agency of the common flagellate, *Trichomonas*, in producing pathological conditions characteristic of blackhead in turkeys, as described in several papers by the present writer, has no legs to go on, and would scarcely receive the consideration of sane protozoologists. Of course this is not the impression that Dr. Tyzzer meant to leave; so that it is fortunate that, in the experimental section of the paper referred to, he makes certain observations which are more favorable to the "flagellate hypothesis." Fearing, however, lest the hypothesis of tissue-invasion by *Trichomonas* might as yet be too frail to survive long under the criticism of two such men as Dr. Tyzzer and Dr. Theobald Smith (formerly chief proponent of the "Amebic theory"), the present writer, who first had the misfortune seriously to mention *Trichomonas* in connection with blackhead, wishes to point out a few instances in which Dr. Tyzzer's criticisms, real or implied, are due either to careless reading of the original papers, or to a too hurried examination of the plates, or to both.

In way of introduction it may be said that Dr. Smith's first exposition of the blackhead disease, together with his original description of the causative agent, *Ameba meleagridis*, appeared in 1895. It is true that, at that time, as Dr. Tyzzer states, the possibility of the relationship between *Ameba meleagridis* and the flagellates was suggested by Dr. Smith. And the suggestion was expressed in these words:

There is probably no genetic relation between this hypothetical organism (flagellate) and the true parasite of the disease under consideration.

For twenty years the "Ameba hypothesis" stood; and it was not until this interpretation was called into question by Cole and the present writer that (as Dr. Tyzzer states), Dr. Smith explained "that the name 'Ameba' is employed tentatively, and

that it may be necessary to change this when the nature of the parasite is better understood." The present writer would probably have been more cautious in his original criticisms of Dr. Smith's conclusions if that statement had been made by Dr. Smith in 1895 instead of 1915. This will answer a criticism of Dr. Tyzzer's that is rather subtle and only implied.

Again, it is implied that something must be wrong with an investigation that purports to demonstrate that *Trichomonas* is the causative agent in an infection, when the investigator can not put his finger on the species concerned, or even risk the foundation of a new species. Who will come forward and give us a clear, definite and usable classification of the Trichomonads! And, speaking of new species in such a poorly known group, one can not help wondering if it would not have been just as well, in an earlier case, to leave the "*meleagridis*" off of *Ameba*. Can one doubt that many unhappy hours and profitless discussions have resulted from the necessity of piling up premature adjectives after an innocent Latin noun? Why embarrass the lexicographers until we are sure? And in the case under consideration the present writer wasn't sure.

Again Dr. Tyzzer states that it is obvious that the present writer "does not consider the organism as primarily pathogenic in nature, but as a normal inhabitant of the alimentary tract of turkeys and fowls which may invade the tissue under conditions which lower the resistance of the host." This is quite true. Then Dr. Tyzzer continues,

Apparently this author attaches no importance to the fact that the disease may be produced in healthy flocks by the introduction of infected birds.

This is also quite true. The writer does not know of a carefully controlled experiment in which it has ever been conclusively demonstrated that blackhead has been produced in healthy flocks by the introduction of infected birds. During one year of experimental work in the field the writer made it a point of removing the ceca and livers of poults which died of blackhead, chopping them up in a meat cutter, mixing lightly with middlings and feeding *en masse* to other poults as a partial substitute for beef-scraps. The mortality from blackhead in the fed group and in the control group was essentially the same. Most of the poults died after several weeks with gape-worm infection, and with no sign of pathologic changes in either ceca or liver. The

writer would have no apprehension in feeding to "healthy" poulters any reasonable amount of pathologic material from blackhead cases, *provided it were done in such a way as not to upset the normal digestive equilibrium, and not to introduce pathogenic bacteria nor bacterial toxins.*

If Dr. Tyzzer had seen as much of blackhead in the field and on the farm as he has seen in the laboratory, he might more readily find reason in the writer's viewpoint. Until a few years ago, the writer held strongly the same views which Dr. Tyzzer now holds. But here, as in some other branches of science, "field work" and field experience has often wholesomely corrected misguided laboratory theory; at least the writer has found it so in his own case.

In another place Dr. Tyzzer states:

Contrary to Hadley's claim *Amœba meleagridis* should not be regarded as a cell parasite. . . . It does not occur within cells except after motility is lost, when it is soon phagocyted.

Regarding the matter of cell-invasion Dr. Tyzzer quotes from a passage from the author but stops prematurely. The passage should be read as a whole to obtain the writers' full meaning. The writer states that, in tissue-invasion, we see *Trichomonas* in a new rôle, and that here it may actively invade living cells. At this point Dr. Tyzzer's quotation stops, but in the original the text proceeds:

One may remark that the type of cell invaded is a highly specialized type [endothelial], and one that, by its nature, is more or less open to invasion.

The writer points out elsewhere that this invasion is not passive but active. But nowhere in any of his published papers (except in reference to the "goblet" cells) does the writer give any expression of the opinion that *Trichomonas* is a cellular parasite in the same sense that applies to the coccidia or other sporozoa. In this respect, Dr. Tyzzer accidentally misrepresents the writer's views. Such little mistakes as always likely to happen in the hurried reading of long and complicated papers.

A further criticism of Dr. Tyzzer's is too good to omit. The circumstances are as follows: The epithelium of the cecum of the turkey is thrown into folds. Sometimes they are deep and sometimes shallow. Within the folds, next to the cecal wall, are the crypts. The projecting folds, with their accompanying tissues,

the writer has referred to as the villi. The author points out that invasion of the submucosa is brought about by the passage of the flagellates through the epithelium of the fundus of the crypt, and that secondarily they invade (from behind) the villi, and finally escape into the cecal lumen after pushing off the epithelium of the villus tips. This phenomenon, which can be followed clearly in suitable sections, the writer has referred to as the stage of "reversed infection," and has pointed out that it constitutes a means whereby the parasites complete their parasitic cycle, rather than being buried and destroyed within the tissues as stated by Dr. Smith, who is of the opinion that the parasite of blackhead lacks this essential feature of perfect parasitism.

Here is Dr. Tyzzer's criticism of this exposition:

The fallacy of such reasoning is quite apparent when the facts of the case are considered. There are no villi in the portion of the cecum commonly involved in blackhead.

The writer had carefully explained in the text the appearance of the invaded tissues; he had pictured it by hand-drawings, and more in detail by a series of photomicrographs. No one could fail to understand the definite histological structure to which the writer referred, whether it is properly termed a "villus," or something else. Dr. Tyzzer may call the histological structure what he pleases. The facts of the case with reference to *Trichomonas* remain the same.

But leaving aside the propriety of the term, villus, let us consider what Dr. Tyzzer means by the balance of his sentence "... in the portion of the cecum commonly involved in blackhead." In the examination of hundreds of cases of blackhead in turkeys and wild fowl the writer has found that blackhead lesions may be initiated anywhere in the cecal wall; there is no part of the cecum that is "commonly involved" except for this circumstance: the majority of the lesions are observed in the distal half of the cecum. Thus Dr. Tyzzer neglects clearly reported facts to grapple with a technical triviality in nomenclature; and at the same time, manifestly from lack of experience with many cases of the disease, misrepresents one of the essential facts relating to cecal infection.

In the next sentence Dr. Tyzzer attacks the statements of the writer regarding the avenue of infection of *Trichomonas*. Re-

ferring to the separation of the epithelium from the basement membrane, he states:

In one case the separation of the epithelium is taken as evidence of invasion, and in the other it is taken as evidence of escape of the flagellates from the tissue.

Dr. Tyzzer quite mistakes the point involved. It is not the separation of the epithelium that is the important point (since this is often an artifact), but the orientation and grouping of the parasites in the vicinity of this epithelium. By looking at a church door we can scarcely tell whether the last congregation went in or out, but if we can find the congregation the question will probably be answered.

And in further criticism of this point (avenue of infection and of exit) Dr. Tyzzer has the misfortune to state,

The organisms interpreted by Hadley as encysted forms of the flagellate being discharged from the tissue are evidently *Blastocystis* derived from the cecal content.

Did Dr. Tyzzer fail to examine the writer's photomicrographs (Bulletin 168, Figs. 30, 32 and 36) together with the complete description of these figures on a preceding page? Did he fail to read the description of this "reversed infection" on page 26? Are the writer's photographs so poor as to make possible a confusion between a flagellate trophozoite and "*Blastocystis*," or has Dr. Tyzzer an inadequate conception of what *Blastocystis* really looks like? And, in addition, may it not be a little inaccurate to affirm that "there is now quite general agreement that they (*Blastocystis*) represent a distinct type of organism . . ."? The matter is apparently still in controversy.

As to the statement of Dr. Tyzzer that the writer has failed to establish the identity of the parasite with any species of *Trichomonas*, or "to demonstrate any features characteristic of the genus,"—this must be left for others to judge. But the author can not forbear to reiterate that he has no reason to withdraw the evidence presented in previous papers. The strongest evidence of all comes from the relatively rare cases in which one can trace from the beginning the movements of the parasites in the tissues, and follow clearly the morphological changes that they undergo as the infection proceeds. It would seem that Dr. Tyzzer, in his examination of only "five infected turkeys," has never seen such cases. The present writer worked for many years before he found the ideal specimens. It is a

hard thing to realize, in such an investigation where one is attempting to ascertain the relation of two widely different entities, that a single average case, even though admirably sectioned and stained, may mean very little. Dozens of cases usually afford a more comprehensive view; and finally one comes to be able to piece together bits of information which make the story clear. It would be miraculous if the keenest pathologist could make clear the evidence from "five cases." Protozoan life histories are not read in a moment, and a study of a hundred cases for an hour means much more than one case for a hundred hours, —unless that one case is exceptional.

In concluding, it may be added that the writer hopes later to consider more in detail the valuable *constructive* aspect of Dr. Tyzzer's paper. It is freely admitted that the life history of *Trichomonas* in the tissues is not wholly clear, and it seems possible that some of the forms referred to by Dr. Tyzzer are new. This is especially true of some of the motile stages which, in the tissues, lose their flagella and, as Schaudinn says, "auch mit stumpflobosen Pseudopodien umherkreicht." It will probably be some years before the last word is said on the blackhead problem; and yet we are progressing. Under an efficient microscope Dr. Tyzzer has given the last blow to the "Amebic theory" and already—though grudgingly—has yielded some support the agency of the flagellates in cecal and liver infections. It may be confidently expected that in the course of time his researches will give more.

Dr. Tyzzer closes his critical introduction with the following words: "It may appear that the above discussion is unduly critical of the findings of other investigators. The confused state of the subject, however, appears to warrant drastic methods and the singling out of various misinterpretations and inconsistencies, for it is quite evident that the enthusiasm of certain investigators for their views has caused them to neglect important facts."

How we all wish to be such champions of the truth! But, in our war on "misinterpretations" and "inconsistencies" and on "neglect of important facts," would not our scientific world be a happier place, and all our work of greater merit, if criticism were tempered more with keen insight and less with the ardent spirit of academic chivalry?

PHILIP HADLEY.

KINGSTON, R. I.,
October 9, 1919

THE INTENSITY OF ASSORTIVE PAIRING IN *CHROMODORIS*¹

THE pairing of the hermaphroditic nudibranch *Chromodoris zebra* is accomplished in such a manner that there occurs a considerable degree of assortive conjugation with respect to size. A report² presenting evidence in support of this conclusion was based upon the examination of *Chromodoris* population in Great Sound, Bermuda, at a season when a considerable percentage of

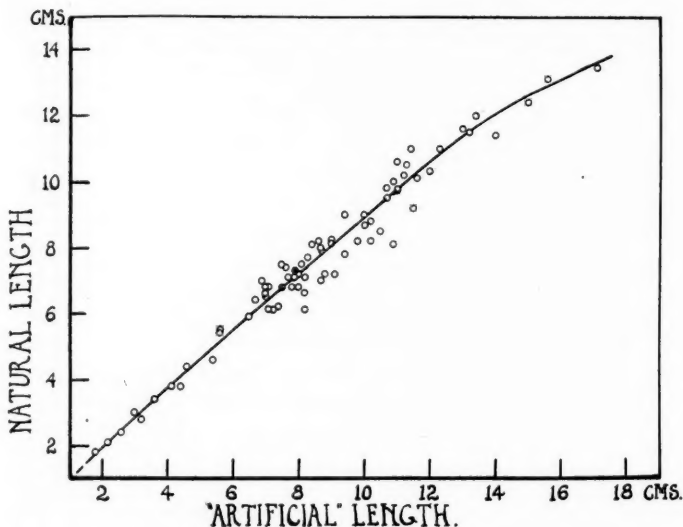


FIG. 1. Curve relating the total length of *Chromodoris* of various sizes to the "length" as obtained from an "artificial" method of measuring the length (see text).

the individuals exhibited injuries of the dorsal region of the mantle. These injuries, resulting in a distortion of the dorsal part of the body, made it necessary in estimating size to measure the total length of the animals—from anterior edge of the buccal veil to posterior termination of the foot. For practical purposes it was necessary at that time to employ a somewhat artificial

¹ Contributions from the Bermuda Biological Station for Research, No. 115.

² Crozier, W. J., "Assortive Mating in a Nudibranch, *Chromodoris zebra* Heilprin," *Jour. Exp. Zool.*, Vol. 27, pp. 247-292, 1918 (cf. *Proc. Nat. Acad. Sci.*, 1917, Vol. 3, pp. 519-522).

method in measuring this length. The animals were placed, dorsal surface downward, upon a glass plate freshly wetted with sea-water. It was recognized² that the soft body of these nudibranchs was by this procedure flattened out, and to some extent increased in length, and that the proportionate amount of distortion might be different for animals of different sizes. Opportunity was therefore subsequently taken to establish the relation

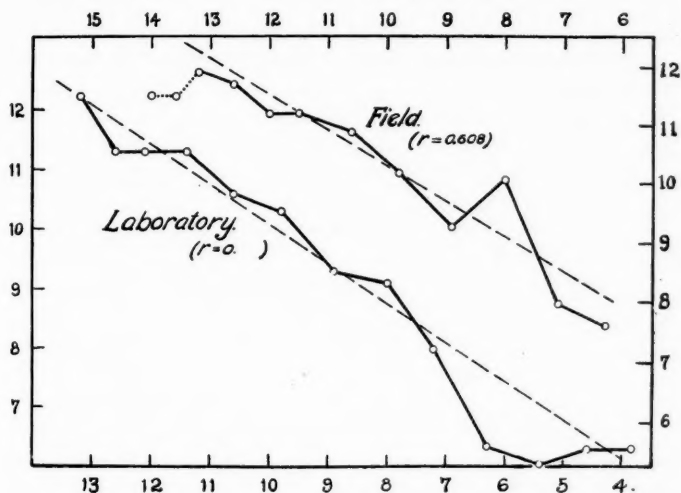


FIG. 2. Regression plots; upper, from 148 pairs copulating in nature; lower, from 119 pairs formed in aquaria; abscissas, length-classes in cms.; ordinates, the mean lengths of the mates of the nudibranchs in each of the corresponding length-classes; length measurements reduced by means of Fig. 1. The regression lines are those previously found (see 2), employing the length as "artificially" determined, and on the assumption that the regression is essentially linear.

between the "artificial" length as previously measured, and the total length of the nudibranch as normally creeping on a flat surface. The lengths of 74 individuals were determined in each of these ways. The result of these measurements is exhibited in Fig. 1.

It is apparent that with nudibranchs of the larger sizes the "normal" length is 1-2 cm. less than the length as artificially estimated; further, that, as was to be expected, the extent of the distortion introduced by the latter method is proportionately greater in larger specimens, the effect being negligible below 3 cm. Accepting the curve as a measure of the relation desired,

Fig. 2 contains regression plots for my data² on pairs found in nature and for laboratory matings in mass experiments, the length-classes having been redistributed according to their respective values in terms of the "normal" length. This procedure involves the assumption that the proportion of flattening in the "artificial" method is the same for animals of the same size-class at different seasons, which is probably not quite exact. The original records were obtained in April-May, 1917, whereas the data for Fig. 1 were secured in September, 1918. In the presence of so many possible sources of variation as these measurements permit, it is sufficient to "average" the determinations graphically, each original length-class, and the corresponding mean length of the mates of individuals in this class, being treated as units in reducing the old "length" figures to the more natural ones obtained through Fig. 1.

According to Fig. 2, the apparent intensity of homogamy in *Chromodoris* is but little affected, if anything perhaps slightly improved, by the reduction of the original figures to the natural scale.

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THE ORIGIN OF THE INTOLERANCE OF INBREEDING IN MAIZE

THE marked intolerance of inbreeding in maize has recently been discussed by Collins¹ and brought to the support of the hypothesis that this plant is of hybrid origin. But to those who look for the origin of maize in another direction, the problem is capable of a very different solution.

Briefly stated, Collins' argument is this: Most varieties of maize suffer from a few generations of self-pollination, but teosinte does not seem to be affected by this treatment. The maize plant as a whole is usually synaemic, with a tendency toward protandry, and self-pollination is in a large degree possible; such inflorescences of maize as have both stamens and pistils are distinctly protogynous. In teosinte the large number of inflorescences on a single plant makes self-pollination a common thing. If maize arose from teosinte, what was the origin of its intolerance of inbreeding? The assumption that maize is

¹ Collins, G. N., "Intolerance of Maize to Self-fertilization," *Jour. Washington Acad. Sci.*, 9: 309-312, 1919.

of hybrid origin takes care of this difficulty by attributing this genetic peculiarity to the unknown parent, which hybridized with teosinte, and in which cross-pollination was probably secured by protogyny.

Three fallacies render this argument inapplicable to the problem that it attempts to solve:

1. We are at once confronted with a question as to how maize, embarrassed by its well-known intolerance of inbreeding and by the extensive self-pollination with which Collins characterizes it, has persisted through the ages. The answer is that self-pollination in the plant is not so common as would be inferred from Collins' discussion.

It is true that a single isolated plant is largely self-pollenized, if pollenized at all, but data derived from the single-stalk culture often practised in experimental work can not be accepted as a criterion, for maize is normally grown in hills, and probably has been for a very long time. This method of cultivation is described by every early explorer and writer on Indian agriculture and seems to have been the rule from the garden beds of the Great Lakes region to the terraced mountain slopes of Peru. In many instances as many as eight or ten plants were grown in a single hill. This was the outgrowth of the Indian's limitations in the way of implements and domesticable animals, and the plant was well adapted to it. The method was adopted by civilized man and is extensively employed, with but few modifications, to the present day.²

If all the plants in a hill were synaemic and flowered at the same time and the air were motionless, the chances for self-pollination would vary inversely as the number of plants in a hill. Tendencies toward protandry, coupled with slight differences in the time of flowering of the individual plants of a hill, the prevalence of winds, and the proximity of other hills increase the chances for cross-pollination. Growing the plants in hills also discourages the production of suckers, thus reducing the number of inflorescences on a single plant, and consequently the chances for self-pollination.

This massing of plants together in hills, and of hills together in fields, is admittedly an artificial element of environment; but its possible evolutionary effect in the ages during which it has

² Cates, H. R., "Farm Practice in the Cultivation of Corn," U. S. Dept. Agr. Bull. 320, 1916, pp. 19-21.

prevailed can not be disregarded. What the condition was in wild maize no one knows, and there is little basis for speculation. But the plant has probably been in cultivation quite long enough to have had its character shaped by agricultural practise.

Directly in accord with this theoretical consideration are Waller's researches,³ which indicate that when corn is grown in hills under ordinary field conditions, self-pollination occurs, on the average, in only a little more than five per cent. of the seeds. As Waller suggests, these figures may be modified by further work on the problem. There is no evidence, however, that the percentage of self-pollination will ultimately be found to be significantly larger than this. The genetic complexity of the average plant selected at random from any ordinary agricultural variety of maize is a standing evidence of the prevalence of cross-pollination.

2. The origin of protogyny in the androgynous inflorescences of maize need not be sought outside the Maydeæ. This is the regular condition in *Tripsacum*, at least in *Tripsacum dactyloides*, which is the only species that I have had opportunity to examine in flower, and it occasionally occurs in *Euchlæna*. Collins⁴ and Kempton⁵ disregard or question the existence of androgynous inflorescences in the latter genus, but the fact of their occasional occurrence remains. The lowest inflorescences of a teosinte plant are almost always wholly pistillate, and the highest wholly staminate. Perfect flowers have not been observed, but between the pistillate and staminate units androgynous inflorescences often occur. These are regularly protogynous. Androgynous inflorescences terminating the main culm are often produced in the greenhouse. The difference between greenhouse plants and those grown in the open in Mexico or southern Florida is fully appreciated. Androgynous terminal inflorescences are certainly of rare occurrence there, if they occur at all; but I am not sure but that they are of less frequent occurrence also in maize grown in tropical or sub-tropical countries. Monœcism in the Maydeæ is readily influenced by environment. The physiological conditions conducive to androgyny in the tassels of maize, and to a relative increase in the number of pis-

³ Waller, A. E., "A Method of Determining the Percentage of Self-pollination in Maize," *Jour. Amer. Soc. Agron.*, 9, 35-37, 1917.

⁴ *Loc. cit.* Also Collins, G. N., "The Origin of Maize," *Jour. Washington Acad. Sci.*, 2: 520-530, 1912.

⁵ Kempton, J. H., "The Ancestry of Maize," *Jour. Washington Acad. Sci.*, 9: 3-11, 1919.

tillate flowers in *Coix* and *Sclerachne*, also produce androgyny in the inflorescence of teosinte. This fact may be of some significance. Maize was doubtless originally a tropical plant. How much of its erratic floral behavior when grown in temperate latitudes is due to real, fundamental differences between it and teosinte, and how much to environment?

It seems, then, that as to androgyny or as to protogyny of the individual inflorescence, there is no fundamental difference between maize and the other American representatives of the Maydææ. When this fact is coupled with a reduction in the number of inflorescences, as in maize, it becomes unnecessary to assume the introduction of the intolerance of self-pollination from another group.

3. Collins leaves in this paper, as well as in an earlier one,⁶ the impression that the alternative to his hybrid origin hypothesis is the theory that maize originated as a mutant from teosinte. The latter idea is quite as chimerical as the former. We can not reasonably hope to find the ancestor of maize in any modern plant; phylogenetic histories seldom work out in this way. The logical procedure is to look for other plants which may have descended, coordinately with maize, from a common ancestor. In *Tripsacum* and *Euchlana* we find two genera that fill the requirements in all known details.⁷

The intolerance of inbreeding in maize is probably the plant's natural evolutionary response to its environment. The maize plant is unique among the grasses in bearing but one pistillate and one staminate inflorescence, or at most only a few inflorescences of each type, in having these widely separated, and in having been grown in hills for untold centuries. These conditions all tend toward extensive cross-pollination, and the data at hand indicate that cross-pollination is the rule. More or less adjustment to these structural characters and this mode of living would be expected; and the decline in vigor, resulting from inbreeding, may be interpreted as the natural consequence of an abnormal and unfavorable condition.

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⁶ Collins, G. N., "Maize: Its Origin and Relationships," *Jour. Washington Acad. Sci.*, 8: 42-43, 1918.

⁷ Weatherwax, Paul, "The Evolution of Maize," *Bull. Torrey Club*, 45: 309-342, 1918.

NOTES AND LITERATURE

Orthogenetic Evolution in Pigeons. Posthumous works of C. O. Whitman, edited by OSCAR RIDDLE. Publication No. 257, Carnegie Inst., Wash. 3 quarto vols. with numerous colored plates and figures. 1919.

In the opening sentence of volume 1 of this notable publication, Whitman says "Progress in science is better indicated by the viewpoints we attain than by massive accumulation of facts." The viewpoint which Whitman himself attained and beyond which he saw no reason for advancing is that of "orthogenesis." His persistent industry also accumulated a mass of facts rarely surpassed in amount concerning variation in a single group of related organisms, the pigeons of the world.

The enormous task of setting these facts in order so as to illustrate his viewpoint, he was unable to accomplish. Death overtook him while he was still busy accumulating facts. But he was fortunate in having a loving pupil willing to devote his life, if necessary, to rescuing from oblivion the work and words of his beloved master. Few literary or scientific executors have shown such self-forgetting devotion or have seen it crowned with such success. Whatever we, living in a period of rapid advance in biology, think at present concerning the value of Whitman's viewpoint, there can be no doubt that Riddle has preserved it permanently, so that no one will be at a loss to know what Whitman's ideas were about the factors of evolution, or on what data they rested.

Whitman took as the point of departure in his pigeon studies, the plumage pattern of the wild rock-pigeon, *Columba livia*, made familiar to everyone by Darwin's use of it in his writings on evolution. Darwin supposed that the wild rock-pigeon of a slate blue color and with two black wingbars was the original form from which all varieties of domestic pigeons had originated through variation and selection. He showed that domestic varieties when intercrossed frequently revert to this wild type and he uses the manifold variation of domestic pigeons as a capital illustration of evolution through descent with modification. Whitman, in the true spirit of science which seeks to "try all things and hold fast [only] that which is good," made inde-

pendent studies of wild rock-pigeons obtained from the "Caves of Cromarty, Scotland." He found that not all the wild pigeons of this locality are of the simple two-wing-bar type, but that part of them show a different pattern known as "chequered." In these also the two black wing-bars can be observed, but they are rendered less conspicuous by the occurrence of other black spots scattered over other parts of the wing, giving the whole a chequered appearance. The wing-bars are due to the occurrence of a black spot on the tip or below the tip of each of two rows of feathers that lie across the wing when it is folded. In chequered birds other rows of feathers bear spots but the spots fall less regularly and obviously into rows, so that the pattern is more like that of a chequer-board. Further in young birds Whitman observed that practically all the wing feathers may bear spots, although in the later plumage some of the spots may disappear. He concluded that *this* condition was the primitive one, rather than the two-wing-bar type which Darwin regarded as primitive. This conclusion seems well founded since the chequered type is thus seen to be less specialized in form and earlier in ontogeny. So far Whitman's work supported Darwin's general evolutionary ideas, merely improving a detail in one of his illustrations, and showing that there still exists among wild pigeons a pattern yet more primitive than the one which Darwin had taken as the point of evolutionary departure. But Whitman now extended his investigations to other species of pigeons and finally to those of the entire world to see if he could work out more fully the evolutionary history of plumage patterns in the group. As a result of these studies he reached conclusions which did not enter into Darwin's scheme of evolution. The most important of these is known by the name of "orthogenesis." This is the idea that evolution through natural selection does not result simply from the selection of chance variations, that variations do not occur in *all* directions but only in particular directions in *straight lines* from the point of departure, hence the name *orthogenesis*. Whitman's study of the plumage patterns of pigeons is probably the most extensive, as it is the most recent, of the studies of a group of animals made in the light of this principle, but to the general body of biologists free from bias for any particular theory it will scarcely be more convincing than its predecessors. It is possible to arrange any group of related organisms in a graded series and to assume that they have been evolved by orderly development, from one end of the series (either end) to the other;

but this is no proof that such has actually been the historic method by which the series has arisen. It may actually have started in the middle and worked both ways, or in several directions. Only a study of contemporaneous genetic variation can show what the method of evolution is. Color variation in mammals is not unlike that of birds. We might arrange the color varieties of any species of mammal or group of mammals in a linear series and assume logically enough that evolution had progressed from the darkest to the lightest form in orderly manner, or *vice versa*, yet the study of contemporaneous variation shows that this is not the case. A wild species, like the gray rabbit or the brown rat, undergoes sporadically genetic variations ("mutations") some of which are lighter, some darker than the parental form. They have no relation to each other as to the order, time, or place of their appearance, so far as we can discover. Breeding evidence shows that they are genetically independent one of another.

As an alternative to the hypothesis of orthogenesis in variation, the mutation theory of DeVries received much critical consideration in Whitman's writings. The lateness of publication of much of this is to be regretted. Discussions which might have been helpful a few years ago are now quite superfluous and out of date in the light of critical experimental evidence since produced.

Mutation has practically ceased to be considered as a hypothetical method of the immediate and direct origin of species. Even as regards the origin of characters, mutation is no longer supposed to be a simple process. Whitman maintains with entire correctness that "unit-characters" often have small beginnings and may later be gradually increased by systematic selection. Frizzling of the feathers in pigeons and fowls is an example cited by him. He says, p. 151:

Minute frills may occur in one or two feathers only, and they may occur in any number, or in all of the feathers. . . . The full character is reached, not by a jump, but by a process of modification, carried farther and farther, from the initial starting point. . . . It is well known that characters often disappear by degrees, not all at once. In crossing species we rarely find the hybrid with *pure* characters. A character may be halved, quartered, etc., to any fractional part of the original.

In passages such as these Whitman clearly shows that the muta-

tion theory as held at that time was untenable when applied either to the origin of species or to the origin of characters. What has since happened is that the mutation theory has been frankly abandoned as applied to such origins and is now limited to the origin of factors or genes. It is recognized that characters may change progressively and permanently (just as Whitman believed they did) under the guidance of selection. The agency of such change is now supposed to be modifying or multiple factors, so numerous as singly almost to baffle detection and so frequently coming and going that gradual modification of characters in a desired direction is not difficult. This is the residuum of truth which underlay the mutation theory as Whitman knew it and attacked it. In this marvellously modified form, he would probably not have attacked the theory at all.

Volume 2 deals chiefly with inheritance, sex, and color in hybrids of wild species of pigeons. An enormous amount of experimental data is here recorded, and scattered notes, briefs for lectures, etc., have been brought together by the editor, dealing with such general topics as heredity, Mendelism, sex determination and the like. As regards the hybrids, only F_1 individuals were produced, for Whitman says, p. 3,

In the case of the wild species of pigeons, of which there are nearly 500, crosses are very often infertile, and fertile hybrids are so rare that Darwin could not find a single well-ascertained instance of hybrids between two true species of pigeons being fertile *inter se*, or even when crossed with one of their pure parents. The records since Darwin's time have not furnished the instance he vainly sought for.

Now every one to-day realizes that the F_2 or second hybrid generation is all important for understanding or interpreting heredity. Whitman accordingly, notwithstanding the boasted superiority as genetic material of the pure species with which he worked, since he was unable to produce in any case a second generation of hybrid birds, had no adequate basis for discussing heredity in his hybrids, and no adequate basis for criticizing Mendelism which is revealed only in the F_2 generation. One characteristic of the large number of sterile F_1 hybrid birds which Whitman produced is noteworthy. Their characters were in nearly all cases blends or intermediates between those of the respective parents. So long as doubt remained as to what the significance of blending is, whether it is essentially different in nature from Mendelian inheritance, Whitman thought rightly

that he had grounds for questioning the universality of Mendelian inheritance. But strong evidence has now been produced that blending inheritance is the regular outcome of crosses involving multiple factorial differences. F_2 in such cases shows increased variability with occasional segregation of the extreme parental types, and in F_3 and F_4 such segregation becomes more common. Had Whitman been able to raise F_2 and F_3 generations, he would undoubtedly have been convinced, contrary to his expectations, as some of us have been, that blending inheritance finds adequate explanation in multiple factor Mendelian inheritance. It is true that Whitman's records of hybrid birds reveal sex-linked inheritance, but these records did not suffice for its discovery, which fell only to those experimenters who worked with the despised "domestic breeds." The most valuable part of the work recorded in this volume is probably the basis which it afforded for experiments on quantitative factors entering into the development and expression of sex, if not its actual determination. This work is due largely to the pupil and editor, Riddle, though he generously brings the name of the master to the front in dealing with the subject. These results have been dealt with more fully in other publications by Riddle and need not here be reviewed.

Volume 3 deals with very different subject matter from that contained in Volumes 1 and 2, viz., the behavior of pigeons. Here is subject matter for the trained animal psychologist and Dr. Riddle felt constrained to call in a competent psychologist to edit this portion of Whitman's writings. Professor H. A. Carr has rendered this important service in a highly acceptable manner. That a single biologist should be able to do distinguished work in two fields so distinct as genetics and animal behavior shows the breadth of Whitman's capacities and interests. The reviewer is unable to deal critically with the contents of Volume 3, but hazards the suggestion that it contains material of very great interest and of permanent value not only to the psychologist but also to the naturalist, the one who is interested in animals as animals rather than as examples and products of one evolutionary process or another.

It is much to be regretted that Professor Whitman was unable himself fully to develop and round out the field of work here so ably outlined and in part explored.

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